BIOAVAILABILITY AND BIOEQUIVALENCE STUDY OF ANTIEPILEPTIC DRUG IN HEALTHY HUMAN VOLUNTEERS

Thesis Submitted *to*

The Tamilnadu Dr.M.G.R Medical University, Chennai

In partial fulfillment of the requirements

for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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SEPTEMBER – 2009

ACKNOWLEDGEMENT

I would like to take this opportunity to place it on record, that this project would never have taken shape but for the cooperation extended to me by all the individuals. Though it is not possible to name all of them, it would be unpardonable on my part if I don't mention some of the very important persons.

I express my sincere thanks to **Dr.R.Venkatanaryana, principal** for granting me the opportunity to do post graduation in pharmacology at this esteemed college.

I consider myself to be very fortunate to have **D.R.Bento Johnson, Head of Dept.of pharmacology** who with his dynamic approach boosted my morale, which helped me in completion of this dissertation.

I express my sincere thanks and gratitude to my institutional guide, **C.Maheswari Assistant Professor,** for her valuable guidance, moral support, and constant encouragement through out this project work.

I owe my sincere gratitude to **Mr. Sunil Reddy**, chairman cum managing director, **BIOSERVE CLINICAL RESEARCH., Pvt, LIMITED**, Balanagar Hyderabad, for giving me a chance to do industrial training in this prestigious organization.

I would like to express my senses of gratitude and sincere thanks to **Mr. Shiva Prasad** Vice President Operations, **BIOSERVE CLINICAL RESEARCH., Pvt, LIMITED,** Hyderabad, for providing me every necessary facilities to carry my project successfully.

I pay my heartiest thanks and gratefulness to my Industrial Guide **Dr. Krishna murthy**, Clinical Investigator, **BIOSERVE CLINICAL RESEARCH., Pvt, LIMITED**,

Hyderabad for his impeccable continual dedicative support to my project work from initiation till final successful completion.

There is no word in my heart to express thanks to Mr.Bhaskar assistant manager in Quality assurance, Miss. Jyothi, Mr. Mahesh, and Mr.Uday who helped me a lot in the entire work.

My sincere thanks to all teaching and non-teaching staff of Department of pharmacology, RVS College of pharmaceutical sciences, who had helped directly or indirectly in the successful completion of my dissertation work.

It gives me an immense pleasure to acknowledge with gratitude, the help rendered by host of people, to whom I owe in a substantial measure in the successful completion of this project.

B.KISHORE KUMAR REDDY

CONTENTS

TABLES LIST

FIGURES LIST

CERTIFICATE

This is to certify that the dissertation work entitled "BIOAVAILABILITY AND BIOEQUIVALENCE STUDY OF ANTIEPILEPTIC DRUG IN HEALTHY HUMAN VOLUNTEERS" is a bonafide work done by Mr.B.KISHORE KUMAR REDDY in partial full fillment of the requirements for the award of MASTER OF PHARMACY IN PHARMACOLOGY and carried out in BIOSERVE CLINICAL RESEARCH COMPANY, HYDERBAD and in the DEPARTMENT OF PHARMACOLOGY, RVS COLLEGE OF PHARMACEUTICAL SCIENCES, SULUR COIMBATORE - 641 402 affiliated to the TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY, under our supervision and guidance.

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CERTIFICATE

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DECLARATION

As required by university regulation, I wish to state that this work

embodied in this thesis titled **"BIOAVAILABILITY AND BIOEQUIVALENCE STUDY OF ANTIEPILEPTIC DRUG IN HEALTHY HUMAN VOLUNTEERS"** forms my own **contribution** to the research work carried out under the guidance of M**iss. Maheshwari** and of **Mr. krishna murthy.** This work has not been submitted for any other degree of this or any other university. When ever references have been made to previous work of others, it has been clearly indicated as such and included in the bibliography.

Signature of the Candidate

Mr. B.KISHORE KUMAR REDDY

1) Overview of the Company[1]

Bioserve clinical Research Private Limited is a **USFDA** certified Contract Research Organization promoted by professionals having over 30 years of experience in the Healthcare and Pharmaceutical industry. It offers a broad range of Clinical Services in compliance with applicable regulatory and ethical guidelines.

Bioserve currently has a 92 Bedded GCP compliant clinical facility (Two independent clinics), capable of undertaking a variety of clinical studies.

Bioserve clinical Research consists of four departments

- Clinical
- Bioanalytical
- Pharmacokinetics and Statistics
- Quality Assurance

Clinical Unit is a 92 Bedded GCP compliant clinical facility (Three independent clinics), capable of undertaking a variety of clinical studies. The Clinical Units are Access Controlled with central monitoring with CCTV attached with well equipped Emergency room and a super specialty hospital, In-house dining and recreation for study participants. Separate areas for Sample Collection, Separation and sample Storage with -40/-80°C deep freezers. Facility to handle light sensitive and other products requiring special

handling. In house canteen with dietician to plan and provide study specific diet to the subjects.

Bioanalytical Unit is fully equipped lab offering HPLC and LCMS-MS analysis with Qualified and experienced team with over 40 years of experience. Access controlled secure sample storage/archival area with -40/-80 ^{0}C deep freezers with Eurotherm 5180V data logger for temperature monitoring. Dedicated sample processing area with all accessories including Positive pressure Solid Phase Extraction (SPE). Our Team is committed to science and meeting the challenges of research by providing seamless outsourcing solutions with professionalism, flexibility and reliability. Our strict compliance to regulatory requirements and our dedication to high quality, accurate results and a commitment to meet sponsor timelines are uncompromising.

Bioserve is also an ISO 9001: 2000 certified facility approved by the Drugs Controller General of India and US-FDA Inspected

Pharmacokinetics and Statistics team is crucial in coordinating clinical trials and a total involvement from design to analysis. The PK group also independently manages and access and temperature controlled pharmacy for the receipt, handling & archival of study drug products in accordance with the regulatory requirements.

Quality Assurance provides expertise to ensure the conduct of client's studies with the highest standards and also conducts independent audits to evaluate compliance with

stipulations of all Standard Operating Procedures and applicable regulatory requirements. QA audits all clinical documents and complete source verification of information. Audits are conducted at regular intervals in order to enable early intervention and necessary corrective action.

2 INTRODUCTION

2.1 General Introduction[2]

Bioavailability can be defined as the degree to which a substance is absorbed or becomes available at the site of physiological activity. It can also be defined as the fraction of an administered dose of medication that reaches the systemic circulation.

When the drug is administered intravenously, its bioavailability is 100%. Where as when a drug is administered via other routes (such as oral route), its bioavailability gets decreased (due to incomplete absorption and first-pass metabolism). Bioavailability is an important tool essential in pharmacokinetics, and is considered to calculate dosages for non-intravenous routes of administration.

Bioavailability reflects the extent of the systemic availability of the active therapeutic moiety and is generally assessed by measuring the 'area under the plasma concentration time curve' (AUC), the peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}).

The extent of the systemic availability is determined by the extent of drug absorbed from the site of administration. For a drug that obeys linear pharmacokinetics, the AUC and C_{max} values increase proportionately with the dose. Consequently, if two formulations / dosage form of the same drug exhibit comparative AUC values, they are considered to have similar systemic

availability. The bioavailability of an oral dosage form or a drug is generally compared with an intravenous solution (100% standard), to determine the absolute bioavailability.

2.2 Bioequivalence

The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

On the basis of simple pharmacokinetic concepts and parameters, bioavailability and bioequivalence studies have been established as acceptable surrogates for expensive, complicated and lengthy clinical trials, and are used extensively worldwide to establish and ensure consistent quality and a reliable, therapeutically effective performance of marketed dosage forms.

Bioequivalence gained increasing attention during the last 40 years after it became evident that generic product and innovator product, having the same amounts of the drug, may exhibit marked differences in their therapeutic responses.

Studies to establish BE of a product are important elements in support of **ANDAs,** of generic drugs.

Generic drug applications are termed "abbreviated" because they are generally not required to include preclinical (animal) and clinical (human) data to establish safety and

effectiveness. Instead, generic applicants must scientifically demonstrate that their product is bioequivalent (i.e., performs in the same manner as the innovator drug). One way scientists demonstrate bioequivalence is to measure the time it takes the generic drug to reach the bloodstream of healthy volunteers. This gives them the rate of absorption, or bioavailability, of the generic drug, which they can then compare to that of the innovator drug. The generic version must deliver the same amount of active ingredients into a patient's bloodstream in the same amount of time as the innovator drug.

Once bioequivalence is established between two products the concept of prescribability and switchability comes into picture. These are concepts about the ease with which the physician can prescribe the generic product or the innovator product depending on various factors.

Drug prescribability is defined as the physician's choice for prescribing an appropriate drug product for his/her new patients between a brand-name drug product and a number of generic drug products of the brand-name product, which have been shown to be bioequivalent to the brand-name drug product. The underlying assumption of drug prescribability is that the brand-name drug product and its generic copies can be used interchangeably in terms of the efficacy and safety of the drug product.

Drug switchability is related to the switch from a drug product (eg: a brand-name drug product) to an alternative drug product (eg: a generic copy of the brand-name drug product) within the same subject whose concentration of the drug product has been

titrated to a steady, efficacious, and safe level. As a result, drug switchability is considered more critical than drug prescribability in the study of drug interchangeability for patients who have been on medication for a while. To assure drug switchability, it is recommended that bioequivalence be assessed within individual subjects. This type of bioequivalence is known as individual bioequivalence (IBE).

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. Concern about lowering health care costs has resulted in tremendous increase in the use of generic drug products; currently about one half of all prescriptions written are for drugs that can be substituted with a generic product.

This phenomenal growth of the generic pharmaceutical industry and the abundance of multisource products have prompted some questions among many health professionals and scientists regarding the therapeutic equivalency of these products. Inherent in the currently accepted guidelines for product substitution is the assumption that a generic drug considered to be bioequivalent to a brand-name drug would elicit the same clinical effect.

The goal of a BE study is to compare the two formulations of same drug by keeping the experimental design and the remaining factors constant.

The extent of the systemic availability is determined by the extent of drug absorbed from the site of administration. For a drug that obeys linear pharmacokinetics, the AUC and C_{max} values increase proportionately with the dose. Consequently, if two formulations / dosage form of the same drug exhibit comparative AUC values, they are considered to have similar systemic availability. The bioavailability of an oral dosage form or a drug is generally compared with an intravenous solution (100% standard), to determine the absolute bioavailability.

2.3 COMPARATIVE BIOAVAILABILITY[3]:

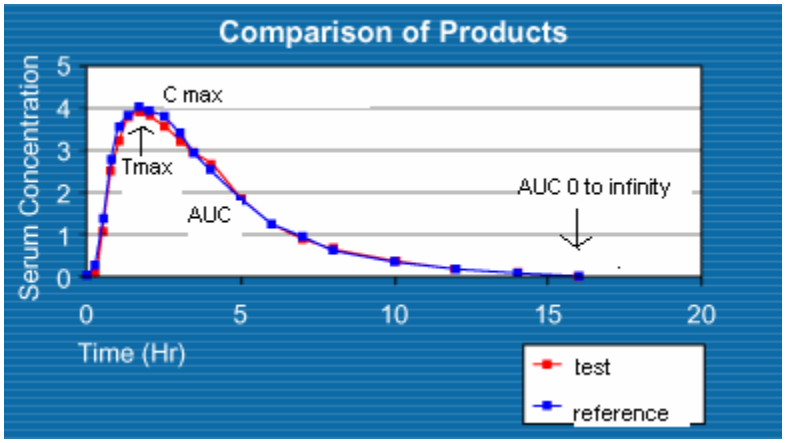
A UNIVERSAL APPROACH:

Most bioavailability studies, whether for a new or generic product, possess a common theme. A test is conducted to identify the quantitative nature of a specific product comparison. This comparison for a new drug may be, for example, to assess the

Performance of an oral formulation relative to that of an intravenous dose, or perhaps the performance of a modified-release formulation in comparison to a conventional capsule.

For a generic product, it is typically a comparison of a competitive formulation with a

reference product. Such commonality surrounding comparative bioavailability studies suggests a universal approach.



- **From the Figure, the two primary metrics for such concentration versus time profiles are the area under the curve (AUC) and the maximum observed concentration (Cmax); the former customarily includes the AUC to the last sampling time in a trial (AUCt) and the extrapolated total AUC to time infinity (AUC∞). The time at which the maximum concentration is obtained (Tmax). After obtaining the profiles in a comparative trial, and Time infinity (AUC∞) is the extrapolated area based on the AUCt and the terminal constant (λz).**

Peak concentration:

Peak concentration should be assessed by measuring the peak drug concentration (C_{max}) obtained directly from the data without interpolation.

- **Total concentration:**

For single-dose studies, the measurement of total concentration should be:

Area under the plasma/serum/blood concentration-time curve from time zero to time t (AUC0-t), where t is the last time point with measurable concentration for individual formulation. Area under the plasma/serum/blood concentration-time curve from time zero to time infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + Ct/\lambda z$, Ct is the last measurable drug concentration and λz. Is the terminal or elimination rate constant calculated according to an appropriate method. The terminal half-life ($t_{1/2}$) of the drug should also be reported.

The following pharmacokinetic parameters are required for submission:

- **Plasma concentrations and time points**
- **Subject, period, sequence, treatment**
- **AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, λz, and $t_{1/2}$.**
- **Intersubject, intrasubject, and/or total variability, if available**

Bioequivalence gained increasing attention during the last 40 years after it became evident that generic product and innovator product, having the same amounts of the drug, may exhibit marked differences in their therapeutic responses.

Situations in which bioequivalence studies are required

- **When significant changes are made in the manufacture of the**

marketed formulation, and

- **When a new generic formulation is tested against the innovator's marketed product.**

Bioequivalence studies compare both the rate and extent of absorption of various multisource formulations with the innovator (reference) product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects.

Bioequivalent simply means that one brand or dosage form of a drug or supplement is equivalent to a reference brand or dosage form of the same drug or supplement in terms of various bioavailability parameters measured via in vivo testing in human subjects.

A product can be either bio-equivalent or bio-in equivalent. A product cannot be more bio-equivalent or less bio-equivalent.

2.4 General Concepts of Design and Conduct of Bioequivalence Studies[4]:

The design and conduct of the study should follow Good Clinical Practice, including reference to an Ethics Committee.

As recommended by the US FDA (1992), in most bioequivalence trials, a "test" formulation is compared with the standard / innovator "reference"

formulation, in a group of normal, healthy subjects (18-55 yr), each of whom receive both the treatments alternately, in a crossover fashion (two-period, two-treatment crossover design), with the two phases of treatment separated by a "washout period" of generally a week's duration, but may be longer (a minimum time equivalent to 5 half-lives) if the elimination half-life of the drug is very long. The treatment is assigned to each subject, randomly, but an equal number of subjects receive (Balanced) each treatment in each phase. Thus, in case of two treatments A and B, one group gets the treatment in the order AB and the second group in the reverse order BA. This is done to avoid the occurrence of possible sequence or period effects. A similar allocation is done in case of a three-treatment crossover design (three-period, three-treatment crossover design).

For several drugs a great inter-subject variability in clearance is observed. The intra-subject coefficient of variation (approximately 15%) is usually substantially smaller than that between subjects (approximately 30%), and therefore, crossover designs are generally recommended for bioequivalence studies.

The primary advantage of the crossover design is that since the treatments are compared on the same subject, the intersubject variability does not contribute to the error variability. If the drug under investigation and/or its metabolites has an extremely long half-life, a parallel group design may be indicated. In a parallel group design, subjects are divided randomly

into groups, each group receiving one treatment only. Thus, each subject receives only one treatment. In a parallel design, although one does not have to worry about sequence, period or carry over effects, or dropouts during the study, the inter-subject variability being very high, the sensitivity of the test is considerably reduced, thus requiring a larger number of subjects compared to a crossover design, to attain the same sensitivity.

Inherent in both the crossover and parallel designs are the three fundamental statistical concepts of study design, namely

- **Randomization**
- **Replication and Error control.**

• Randomization

It implies allocation of treatments to the subjects without selection bias. Consequently, randomization is essential to determine an unbiased estimate of the treatment effects.

• Replication

It implies that a treatment is applied to more than one experimental unit (subject) to obtain more reliable estimates than is possible from a single observation and hence provides a more precise measurement of treatment effects. The number of replicates (sample size) required will depend upon the

degree of differences to be detected and inherent variability of the data. Replication is used concomitantly with "Error control" to reduce the experimental error or error variability.

Crossover design for two medications (T–test; R = reference):

2x2 crossover design:

This is a conventional not-replicated design with two formulations, two periods, two sequences, each individual is randomly assigned to RT or TR sequence in two dosage periods. That is, individuals assigned to RT (TR) sequence receive formulation R (T) in the first dosage period and formulation T (R) in the second dosage period. Randomization for a 2x2 crossover study may be carried out through tables of random numbers or randomization procedures implemented by statistical software. It is represented as follows:

Sequence	Period	
	1	2
1	R	T
2	T	R

Replicated crossover design:

More commonly used replicated crossover designs to compare two formulations are:

Four-sequence and two-period design (Balaam's design):

Sequence	Period	
	1	2
1	T	T
2	R	R
3	R	T

4	T	R

Two-sequence and four-period design:

Sequence	Period			
	1	2	3	4
1	T	R	R	T
2	R	T	T	R

Four-sequence and four-period design:

Sequence	Period			
	1	2	3	4
1	T	T	R	R
2	R	R	T	T
3	T	R	R	T
4	R	T	T	R

Two-sequence and three-period design:

Sequence	Period		
	1	2	3
1	T	R	T
2	R	T	R

Crossover design for three medications (Williams' design):

(Williams' design with T1 = test 1, T2 = test 2, R = reference)

In order to compare three formulations of a drug, there are a total of three possible comparison pairs among formulations: formulation 1 versus formulation 2, formulation 1 versus formulation 3, and formulation 2 versus formulation 3.

Sequence	Period		
	1	2	3
1	R	T2	T1
2	T1	R	T2
3	T2	T1	R
4	T1	T2	R
5	T2	R	T1
6	R	T1	T2

Crossover design for four medications (Williams' design):

This design is recommended for bioequivalence studies of formulations with modified-release dosage or highly variable products (intra-individual variation coefficient ≥30%), including the quick-release, and modified-release ones and other oral administration products.

The same test and reference formulation batches shall be used for this design for replicated administration. The periods shall be sufficiently spaced (washout) to assure non-existence of carryover effects.

Sequence	Period			
	1	2	3	4
1	R	T3	T1	T2
2	T1	R	T2	T3
3	T2	T1	T3	R
4	T3	T2	R	T1

- **Wash out period:**

Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (wash-out period). Sampling should be done long enough to cover at least 80% of the area under the plasma concentration curve as extrapolated to infinity. The extrapolation should be based on knowledge of the dominating elimination half-life.

- **Subjects:**

The number of subjects required is determined by the error variance associated with the primary characteristic to be studied (as estimated from a pilot experiment, from previous studies or from published data), by the significance level desired, and by the deviation from the reference product compatible with bioequivalence and with safety and efficacy. It should be calculated by appropriate methods and should not be smaller than 12. The deviation allowable usually is. 20%. The number of recruited subjects should always be justified.

Bioavailability studies generally will be performed with healthy volunteers. If feasible, they should belong to both sexes and be between 18 and 50 years old to minimize intra- and inter-individual variation subjects should be standardized as much as possible and acceptable. They should preferably be fasting at least 10 hours during the night before administration of the products or they should take a standard meal at a specified time before the treatment. Time and preferably composition of meals taken after the treatment should be standardized. Because fluid intake may profoundly influence

gastric passage, it should be strictly standardized and specified. The subjects should not take other medicines during a suitable period before and during the study. They should preferably abstain from food and drinks, which may interact with circulatory, gastro-intestinal, liver or renal function (e.g. alcoholic or xanthine-containing beverages). Preferably they should be non-smokers. If smokers are included they should be identified as such. In some cases (e.g. study of high clearance substances) even posture or physical activity may have to be standardized.

- **Reference and test product:**

All investigated products must have been prepared in accordance with GMP-rules. Batch control results of the test product should be reported. Generic products, being pharmaceutical equivalents or alternatives are normally compared with the corresponding form of a well-established "Innovator" medicinal product (reference product). The applicant should justify the choice of reference product. The test product will mostly originate from a test batch. After scale-up samples of the product the production batches should be compared with those of the test batch, and they should show the same dissolution rate "in vitro" in a discriminatory test. The study sponsor will have to retain a sufficient number of product samples for the accepted shelf life plus one year to allow repetition of "in vitro" and "in vivo" studies at the request of the regulatory authority.

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- **Data analysis:**

The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant. The primary concern in bioequivalence assessment is to limit the risk of erroneously accepting bioequivalence. Only statistical procedures, which do not exceed the nominal risk of 5%, can be approved, and among them the one with the smallest risk of erroneously rejecting bioequivalence should be selected.

In case of a parametric approach the inclusion of the classical 90% confidence interval for the chosen measure of relative bioavailability within the acceptance range (bioequivalence range) is the procedure of choice. This procedure is equivalent to the rejection of two one sided hypotheses concerning bioinequivalence at the nominal 5%-level. According to present views concentrations and concentration-related characteristics (e.g. AUC, MRT) should preferably be tested after logarithmic transformation. If the assumption of a log normal (AUC, C_{max}) distribution or normal (t_{max}) distribution in the parametric approach is doubtful, a corresponding non-parametric approach is

recommended. This approach may also be chosen as the general statistical approach to evaluate all bioavailability characteristics throughout a given study. Assumptions on the design or statistical analysis should be discussed.

- **"In vitro" Dissolution**

The results of "in vitro" dissolution tests, obtained with the batches of test and reference products that are used in the bioavailability or bioequivalence study. The specifications for the "in vitro" dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioavailability or bioequivalent.

- **Reporting**

The report of a bioavailability or bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP. This implies that the signature of the study monitor attests the authenticity of the whole of the report. The responsible investigators should sign for their respective sections of the report. Names and affiliations of the responsible investigators, site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s) of the test product(s) should be given. In addition the applicant may submit a signed statement, confirming the identity of the test product with the product, which is submitted for registration. All results should be presented in a clear way. The way of calculating the characteristics used (e.g. AUC) from the raw data should be specified. Deletion of data should be justified. If results are calculated using pharmacokinetic models the model and the computing procedure used should be justified.

Individual plasma concentration/time curves should be drawn on a linear/linear, and facultative also on a linear/log scale. The report should contain individual data and results, it should also give the details, of dropped-out subjects.

A representative number of chromatograms should be included. The analytical validation report should be reported.

2.5 AN OVERVIEW OF THE DISEASE

Epilepsy is a neurological condition that from time to time produces brief disturbances in the normal electrical functions of the brain. Normal brain function is made possible by millions of tiny electrical charges passing between nerve cells in the brain and to all parts of the body. When someone has epilepsy, this normal pattern may be interrupted by intermittent bursts of electrical energy that are much more intense than usual. They may affect a person's consciousness, bodily movements or sensations for a short time.

These physical changes are called epileptic seizures. That is why epilepsy is sometimes called a seizure disorder. The unusual bursts of energy may occur in just one area of the brain (partial seizures), or may affect nerve cells throughout the brain (generalized seizures). Normal brain function cannot return until the electrical bursts subside. Conditions in the brain that produce these episodes may have been present since birth, or they may develop later in life due to injury, infections, structural abnormalities in the brain, exposure to toxic agents, or for reasons that are still not well understood. Many illnesses or severe injuries can affect the brain enough to produce a single seizure. When seizures continue to occur for unknown reasons or because of an underlying problem that

cannot be corrected, the condition is known as epilepsy. Epilepsy affects people of all ages, all nations, and all races. Epilepsy can also occur in animals, including dogs, cats, rabbits, and mice.

Epilepsy is a neurological disorder that affects people in every country throughout the world. Epilepsy is also one of the oldest conditions known to mankind. It is characterized by a tendency to recurrent seizures and it defined by two or more unprovoked seizures.

A Seizure or convulsion is a brief period of unconsciousness or altered consciousness. It may be accompanied by one or more of the following symptoms: falling; muscle spasms; drooling or "frothing" at the mouth, loss of bladder or bowel control, a temporary halt in breathing.

Seizures may occur in anyone, irrespective of the age. It can be a terrifying sight when a person suffers a seizure and most often the family members or attendants of the person do not know what to do. A seizure struck person does not respond. Panic and alarm are the first reactions but an understanding of the underlying mechanism can alleviate anxiety.

CAUSES:

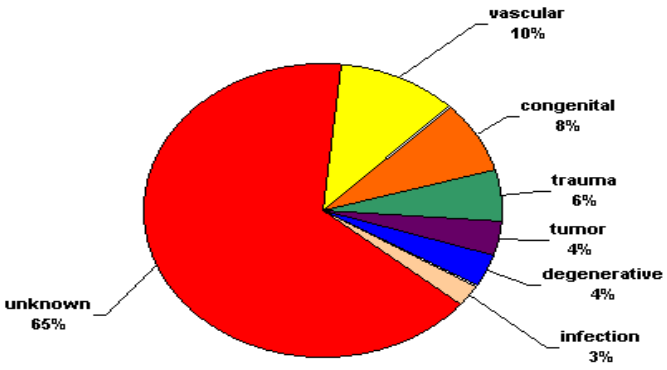


Figure-1 causes of epilepsy[5]

Our brain is an enormously huge and complex network of electrical circuits. Seizures are the result of abnormal activity in one area of this circuit which causes abnormal currents to spread to the rest of the brain. The result is a seizure with its attendant physical and/or behavioral manifestations.

Seizures are associated with many medical conditions. Most convulsions in infants and toddlers are caused by fever. Though they are terrifying to parents, these seizures are usually brief and rarely cause lasting damage. Seizures may also be caused by gastrointestinal disease, poisoning, head injury, brain disease such as a tumor, and rarely, breath-holding during a tantrum. Repeated convulsions might turn out to be an indicator to a chronic condition of epilepsy.

Definition[6]

"A seizure is a sudden change in behavior due to abnormal electrical activity in the brain". A Seizure or convulsion is a brief period of unconsciousness or altered consciousness. It may be accompanied by one or more of the following symptoms: falling; muscle spasms; drooling or "frothing" at the mouth, loss of bladder or bowel control, a temporary halt in breathing.There are so many kinds of seizures that neurologists who specialize in epilepsy are still updating their thinking about how to classify them.

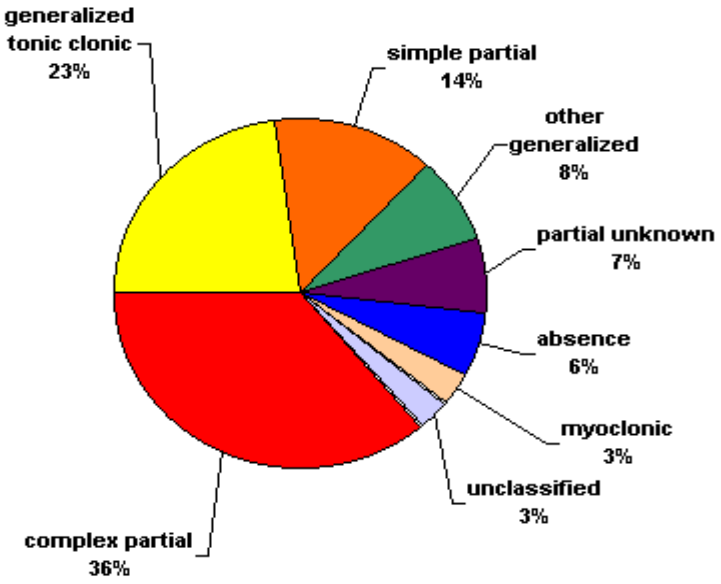


Figure -2 Types of seizures[5]

Usually, they classify seizures into two types, *primary generalized* seizures and *partial seizures*.

The difference between these types is in how they begin:

2.5.1 Primary generalized seizures

Primary generalized seizures begin with a widespread electrical discharge that involves both sides of the brain at once. Hereditary factors are important in many of these seizures.

2.5.2 Partial seizures

Partial seizures begin with an electrical discharge in one limited area of the brain. Some are related to head injury, brain infection, stroke, or tumor, but in most cases the cause is unknown.

One question that is used to further classify partial seizures is whether consciousness (the ability to respond and remember) is "impaired" or "preserved." The difference may seem obvious, but really there are many degrees of impairment or preservation of consciousness. Identifying certain seizure types and other characteristics of a person's epilepsy like the age at which it begins, for instance, allows doctors to classify some cases into epilepsy syndromes. This kind of classification helps us to know how long the epilepsy will last and the best way to treat it.

Primary Generalized seizures are further classified into following types

a) Absence seizures

b) Atypical absence seizures

c) Myoclonic seizures

d) Atonic seizures

e) Tonic seizures

f) Clonic seizures

g) Tonic-clonic seizures

Partial Seizures are further classified into following types

i. Simple partial seizures

ii. Complex partial seizures

iii. Secondarily generalized seizure

Primary Generalized seizures

a. Absence seizures:

Usually less than 10 seconds, but it can be as long as 20 seconds. They begin and end suddenly. Absence seizures are brief episodes of staring. Another name for them is petit mal. During the seizure, awareness and responsiveness are impaired. People who have them usually don't realize when they've had one. There is no warning before a seizure, and the person is completely alert immediately afterward. ***Simple*** **absence** seizures are just stares. Many absence seizures are considered as ***complex*** **absence** seizures, which mean that they include a change in muscle activity. The most common movements are eye blinks. Other movements include slight tasting movements of the mouth, hand movements such as rubbing the fingers together, and contraction or relaxation of the muscles. Complex absence seizures are often more than 10 seconds long.

Absence seizures usually begin between ages 4 and 14. The children who get them usually have normal development and intelligence. In nearly 70% of cases, absence seizures stop by age 18. Children who develop absence seizures before age 9 are much more likely to outgrow them than children whose absence seizures start after age 10. Children with absence seizures do have higher rates of behavioral, educational, and social problems.

b. Atypical Absence

Usually 5 to 30 seconds (commonly more than 10), with a gradual beginning and ending.

Atypical means unusual or not typical. The person will stare (as they would in any absence seizure) but often is somewhat responsive. Eye blinking or slight jerking movements of the lips may occur. This behavior can be hard to distinguish from the person's usual behavior, especially in those with cognitive impairment. Unlike other absence seizures, these seizures usually cannot be produced by rapid breathing.

They generally begin before age 6. Most of the children affected have below-average intelligence and other types of seizures that are difficult to control. Many have Lennox-Gastaut syndrome.

Atypical absence seizures usually continue into adulthood. Sometimes ordinary behavior for these children will look like an atypical absence seizure. Daydreaming and inattentiveness can mimic these seizures.

c. Myoclonic Seizures

They're very brief jerks. Usually they don't last more than a second or two. There can be just one, but sometimes many will occur within a short time.

The epileptic syndromes that most commonly include myoclonic seizures usually begin in childhood, but the seizures can occur at any age. Other characteristics depend on the specific syndrome.

Myoclonic seizures are brief, shock-like jerks of a muscle or a group of muscles. "Myo" means muscle and "clonus" means rapidly alternating contraction and relaxation—jerking or twitching—of a muscle. Even people without epilepsy can experience myoclonus in hiccups or in a sudden jerk that may wake you up as you're just falling asleep. These things are normal.

In epilepsy, myoclonic seizures usually cause abnormal movements on both sides of the body at the same time. They occur in a variety of epilepsy syndromes that have different characteristics:

- *Juvenile myoclonic epilepsy*: The seizures usually involve the neck, shoulders, and upper arms. In many patients the seizures most often occur soon after waking up. They usually begin around puberty or sometimes in early adulthood in people with a normal range of intelligence. In most cases, these seizures can be well controlled with medication but it must be continued throughout life.

- *Lennox-Gastaut syndrome:* This is an uncommon syndrome that usually includes other types of seizures as well. It begins in early childhood. The myoclonic

seizures usually involve the neck, shoulders, upper arms, and often the face. They may be quite strong and are difficult to control.

- *Progressive myoclonic epilepsy:* The rare syndromes in this category feature a combination of myoclonic seizures and tonic-clonic seizures. Treatment is usually not successful for very long, as the patient deteriorates over time.

As mentioned, some episodes of myoclonus are normal. Some myoclonic seizures occur in reflex epilepsies, triggered by flashing lights or other things in the environment.

d. Atonic Seizures

Duration is less than 15 seconds.

Muscle "tone" is the muscle's normal tension. "Atonic" means "without tone," so in an atonic seizure, muscles suddenly lose strength. The eyelids may droop, the head may nod, and the person may drop things and often falls to the ground. These seizures are also called "drop attacks" or "drop seizures." The person usually remains conscious.

Another name for this type of seizure is "akinetic", which means "without movement."Atonic seizures often begin in childhood. They often last into adulthood.

e. Tonic Seizures

Usually less than 20 seconds.

Muscle "tone" is the muscle's normal tension at rest. In a "tonic" seizure, the tone is greatly increased and the body, arms, or legs make sudden stiffening movements. Consciousness is usually preserved. Tonic seizures most often occur during sleep and usually involve all or most of the brain, affecting both sides of the body. If the person is standing when the seizure starts, he or she often will fall.

They are particularly common in people who have the epilepsy syndrome called Lennox-Gastaut syndrome, but they can occur in anyone. Tonic seizures in Lennox-Gastaut syndrome may become more difficult to control over time. Some patients do achieve a good outcome. Children with neurological impairments sometimes make movements that could be mistaken for tonic seizures.

f. Clonic Seizures

Clonic seizures consist of rhythmic jerking movements of the arms and legs, sometimes on both sides of the body. The length of time varies.

"Clonus" means rapidly alternating contraction and relaxation of a muscle - in other words, repeated jerking. The movements cannot be stopped by restraining or repositioning the arms or legs. Clonic seizures are rare, however. Much more common are tonic-clonic seizures, in which the jerking is preceded by stiffening (the "tonic" part). Sometimes tonic-clonic seizures start with jerking alone. These are called clonic-tonic-clonic seizures.

Clonic seizures are not seen very often. They can occur at various ages, including in newborns. Brief and infrequent clonic seizures in infants usually disappear on their own within a short time. Other types may need prolonged treatment.

Occasionally "jitteriness" in a young infant can be mistaken for a clonic seizure, especially if it is severe (during crying, for instance). Changing the position of the baby's arms or legs should reduce or stop jitteriness. The jittery infant also will be more alert than an infant who is having a clonic seizure. Children with neurological impairments sometimes have repetitive movements that could be mistaken for clonic seizures.

g. Tonic-Clonic Seizures

Generally, 1 to 3 minutes. A tonic-clonic seizure that lasts longer than 5 minutes probably calls for medical help. A seizure that lasts more than 30 minutes, or three seizures without a normal period in between, indicates a dangerous condition called convulsive status epilepticus. This requires emergency treatment.

This type is what most people think of when they hear the word "seizure." An older term for them is "grand mal." As implied by the name, they combine the characteristics of tonic seizures and clonic seizures. The tonic phase comes first. All the muscles stiffen. Air being forced past the vocal cords causes a cry or groan. The person loses consciousness and falls to the floor. The tongue or cheek may be bitten, so bloody saliva may come from the mouth. The person may turn a bit blue in the face. After the tonic phase comes the clonic phase: The arms and usually the legs begin to jerk rapidly and rhythmically, bending and relaxing at the elbows, hips, and knees. After a few minutes, the jerking slows and stops. Bladder or bowel control sometimes is lost as the body

relaxes. Consciousness returns slowly, and the person may be drowsy, confused, agitated, or depressed. They affect both children and adults.

For children who have had a single tonic-clonic seizure, the risk that they will have more seizures depends on many factors. Some children will outgrow their epilepsy. Often, tonic-clonic seizures can be controlled by seizure medicines. Some nonepileptic (psychogenic) seizures resemble tonic-clonic seizures. The surest way to tell the difference is with video-EEG monitoring. In some cases, the same person may have both tonic-clonic and nonepileptic seizures.

People who faint sometimes develop tonic or clonic movements. These movements are rarely as intense or prolonged as a tonic-clonic seizure.

PARTIAL SEIZURES

i. Simple Partial Seizures

Duration is usually less than 2 minutes.

They are remarkably different from person to person, depending on the part of the brain where they begin. The one thing they all have in common is that the person remains alert and can remember what happens. Here are a couple of experiences:

- *"It is a pressure that starts in my stomach, then rises to my chest and throat. When it reaches my chest, I smell an unpleasant odor of something burnt. At the same time I feel anxious."*

Sometimes the seizure activity spreads to other parts of the brain, so another type of seizure follows the simple partial seizure. This can be a complex partial seizure or a secondarily generalized seizure.

Doctors often divide simple partial seizures into categories depending on the type of symptoms the person experiences:

Motor seizures:

These cause a change in muscle activity. For example, a person may have abnormal movements such as jerking of a finger or stiffening of part of the body. These movements may spread, either staying on one side of the body (opposite the affected area of the brain) or extending to both sides. Other examples are weakness, which can even affect speech, and coordinated actions such as laughter or automatic hand movements. The person may or may not be aware of these movements.

Sensory seizures:

These cause changes in any one of the senses. People with sensory seizures may smell or taste things that aren't there; hear clicking, ringing, or a person's voice when there is no actual sound; or feel a sensation of "pins and needles" or numbness. Seizures may even be painful for some patients. They may feel as if they are floating or spinning in space. They may have visual hallucinations, seeing things that aren't there (a spot of light, a scene with people). They also may experience illusions—distortions of true sensations. For instance, they may believe that a parked car is moving farther away, or that a person's voice is muffled when it's actually clear.

Autonomic seizures:

These cause changes in the part of the nervous system that automatically controls bodily functions. These common seizures may include strange or unpleasant sensations in the stomach, chest, or head; changes in the heart rate or breathing; sweating; or goose bumps.

Psychic seizures:

These seizures change how people think, feel, or experience things. They may have problems with memory, garbled speech, and an inability to find the right word, or trouble understanding spoken or written language. They may suddenly feel emotions like fear, depression, or happiness with no outside reason. Some may feel as though they are outside their body or may have feelings of déja vu ("I've been through this before") or jamais vu ("This is new to me"— even though the setting is really familiar).

Anybody can get them. They may be more likely in people who have had a head injury, brain infection, stroke, or brain tumor but most of the time the cause is unknown.

Medical disorders such as, stomach disorders or a pinched nerve can cause some similar symptoms. Hallucinations can accompany psychiatric illness or the use of certain drugs. And some symptoms (such as déja vu) are experienced by almost everyone at some time. Whether the symptoms represent simple partial seizures depends on how often they occur and whether they are associated with other episodic changes or other seizure types.

ii. Complex Partial Seizures

They usually last between 30 seconds to 2 minutes. Afterward, the person may be tired or confused for about 15 minutes and may not be fully normal for hours.

These seizures usually start in a small area of the temporal lobe or frontal lobe of the brain. They quickly involve other areas of the brain that affect alertness and awareness.

So even though the person's eyes are open and they may make movements that seem to have a purpose, in reality "nobody's home." If the symptoms are subtle, other people may think the person is just daydreaming.

Some people can have seizures of this kind without realizing that anything has happened. Because the seizure can wipe out memories of events just before or after it, however, memory lapses can be a problem.

Some of these seizures (usually ones beginning in the temporal lobe) start with a simple partial seizure. Also called an aura, this warning seizure often includes an odd feeling in the stomach. Then the person loses awareness and stares blankly. Most people move their mouth, pick at the air or their clothing, or perform other purposeless actions. These movements are called "automatisms" (aw-TOM-ah-TIZ-ums). Less often, people may repeat words or phrases, laugh, scream, or cry. Some people do things during these seizures that can be dangerous or embarrassing, such as walking into traffic or taking their clothes off. These people need to take precautions in advance.

Complex partial seizures starting in the frontal lobe tend to be shorter than the ones from the temporal lobe. The seizures that start in the frontal lobe are also more likely to include automatisms like bicycling movements of the legs or pelvic thrusting. Some complex partial seizures turn into secondarily generalized seizures.

Anybody can get them. They may be more likely in people who have had a head injury, brain infection, stroke, or brain tumor but most of the time the cause is unknown.

As for many other kinds of seizures, the outlook depends on whether the cause is known. They may be outgrown or controlled with medication. If medication is not effective, some can be eliminated by epilepsy surgery.

iii. Secondarily Generalized Seizures

Seizures of this kind start as a partial seizure—that is, they start in one limited area of the brain. The forms they take vary as much as other partial seizures. But then (sometimes so quickly that the partial seizure is hardly noticed) the seizure spreads throughout the brain, becoming "generalized."

The generalized, convulsive phase of these seizures usually lasts no more than a few minutes, the same as primary generalized seizures. The preceding partial seizure is usually not very long. Sometimes this part is so brief that it is hard to detect.

These seizures are called "secondarily generalized" because they only become generalized (spread to both sides of the brain) after the initial or "primary" event, a partial seizure, has already begun. They happen when a burst of electrical activity in a limited area (the partial seizure) spreads throughout the brain. Sometimes the person does not recall the first part of the seizure. These seizures occur in more than 30% of people with partial epilepsy. They can affect people of all ages who have partial seizures.

Many seizures of this kind can be controlled with medication. If a person has tonic-clonic seizures that are not well controlled with medication, the doctor should investigate to see whether they might be secondarily generalized seizures that begin in a limited area of the brain. If they are, surgery could be an option.

It may be difficult to distinguish these seizures from primary generalized tonic-clonic seizures, especially if they occur during sleep or are not witnessed by anyone else. Most convulsive seizures during sleep are secondarily generalized seizures that do begin as partial seizures.

2.6 Epidemiology:

Epilepsy knows no geographical, racial or social boundaries. It occurs in men and women and can begin at any age, but is most frequently diagnosed in infancy, childhood, adolescence and old age. Anyone can be affected by seizures. In fact, up to 5% of the world's population may have a single seizure at some time in their lives, but a diagnosis of epilepsy is reserved for those who have recurring seizures, at least two unprovoked ones.

Prevalence

The prevalence of a disorder is the proportion of a population with that disorder at a given point in time. From many studies around the world it has been estimated that the mean prevalence of active epilepsy (i.e. continuing seizures or the need for treatment) is approximately 8.2 per 1,000 of the general population. However, this may be an underestimate as some studies in developing countries (such as Colombia, Ecuador, India, Liberia, Nigeria, Panama, United Republic of Tanzania and Venezuela) suggest a prevalence of more than 10 per 1,000.

Thus, it is likely that around 50 million people in the world have epilepsy at any one time. The lifetime prevalence of epilepsy (i.e. the number of people presently in the

world who have epilepsy now or have had it in the past or will experience it in the future) is approximately 100 million people.

Incidence

The incidence of a disorder is the number of new cases at a given time. Studies in developed countries suggest an annual incidence of epilepsy of approximately 50 per 100,000 of the general population. However, studies in developing countries suggest that this figure is nearly double that at 100 per 100,000.

One of the main reasons for the higher incidence of epilepsy in developing countries is the higher risk of experiencing a condition which can lead to permanent brain damage. These conditions include [1]neurocysticercosis, meningitis, malaria, pre and perinatal complications and malnutrition.

Mortality

Epilepsy is associated with an increased risk of mortality. Death may be related to:

- An underlying brain disease, such as a tumor or infection.
- Seizures in dangerous circumstances, leading to drowning, burns or head injury.
- Status epilepticus.
- Sudden and unexplained causes or a possible respiratory or cardio-respiratory arrest during a seizure.
- Suicide.

Diagnosis

A seizure disorder can be diagnosed when people have at least two unprovoked seizures that occur at different times. The diagnosis is based on symptoms and the observations of eyewitnesses. Symptoms that suggest a seizure include loss of consciousness, muscle spasms that shake the body, loss of bladder control, sudden confusion, and inability to pay attention. However, seizures cause such symptoms much less often than most people think. A brief loss of consciousness is more likely to be fainting (syncope—see Low Blood Pressure: Fainting) than a seizure.

An eyewitness report of the episode can be very helpful to doctors. An eyewitness can describe exactly what happened, whereas people who have an episode usually cannot. Doctors need to have an accurate description, including the following:

- How fast the episode started
- Whether it involved abnormal muscle movements (such as spasms of the head, neck, or facial muscles), tongue biting, drooling, loss of bladder control, or muscle stiffening
- How long it lasted
- How quickly the person recovered

Although eyewitnesses may be too frightened during the seizure to remember all details, whatever they can remember can help. If possible, how long a seizure lasts should be

timed with a watch or other device. Seizures that last only 1 or 2 minutes can seem to go on forever.

There is a need to know what people experienced before the episode: whether they had a premonition or warning that something unusual was about to happen and whether anything, such as certain sounds or flashing lights, seemed to trigger the episode. Doctors ask whether people have had a disorder that can cause seizures (such as a brain infection) or a head injury. Doctors also ask about which drugs (including alcohol) people are taking or have recently stopped. A thorough physical examination is done. It may provide clues to the cause of the symptoms.

People are usually evaluated in an emergency department. If a seizure disorder has already been diagnosed and people have completely recovered, they may be evaluated in a doctor's office.

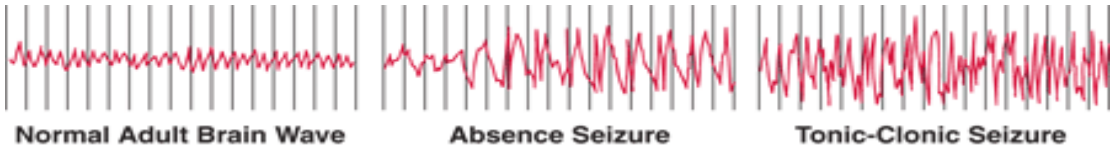
Once a seizure is diagnosed, more tests are usually needed to identify the cause. People known to have a seizure disorder may not need additional tests. In others, blood tests are often done to measure the levels of substances such as sugar, calcium, sodium, and magnesium and to determine whether the liver and kidneys are functioning normally. A sample of urine may be analyzed to check for recreational drugs that may not be reported. Such drugs can trigger a seizure. Electrocardiography may be done to check for an abnormal heart rhythm. Because an abnormal heart rhythm can greatly reduce blood flow to the brain, it can trigger loss of consciousness and occasionally a seizure or symptoms that resemble a seizure.

Computed tomography (CT) is usually done promptly to check for bleeding, tumors, and other structural damage to brain tissue (for example, by a stroke). If results are negative, magnetic resonance imaging (MRI) is usually done later. It provides detailed images of abnormalities and can detect most neurologic disorders.

Electroencephalography (EEG) can help confirm the diagnosis. EEG is a painless, safe procedure that records electrical activity in the brain. Doctors examine the recording (electroencephalogram) for evidence of abnormal electrical discharges. Because the recording time is limited, EEG can miss abnormalities, and results may be normal, even in people who have a seizure disorder. EEG is sometimes scheduled after people have been deprived of sleep for 18 to 24 hours because lack of sleep makes abnormal discharges more likely to occur.

Brain Activity during a Seizure

An electroencephalogram (an EEG) is a recording of the brain's electrical activity. The procedure is simple and painless. About 20 small adhesive electrodes are placed on the scalp, and the brain's activity is recorded under normal conditions. Then the person is exposed to various stimuli, such as bright or flashing lights, to try to provoke a seizure. During a seizure, electrical activity in the brain accelerates, producing a jagged wave pattern. Such recordings of brain waves help identify a seizure disorder. Different types of seizures have different wave patterns.



If the diagnosis is still uncertain, specialized tests, such as video-EEG monitoring, can be done at an epilepsy center. For this test, people are admitted to a hospital for 2 to 7 days, and EEG is done while they are video-taped. If people are taking an anticonvulsant, it is often stopped to increase the likelihood of a seizure. If a seizure occurs, doctors compare the EEG recording with the video recording of the seizure. They may then be able to identify the type of seizure and the area of the brain where the seizure began.

Treatment

If the cause can be identified and eliminated, no additional treatment is necessary. For example, if a low blood sugar (glucose) level (hypoglycemia—see Hypoglycemia) caused the seizure, glucose is given, and the disorder causing the low level is treated. Other treatable causes include an infection, certain tumors, and an abnormal sodium level.

If people have a seizure disorder, general measures plus drugs are usually sufficient. If drugs are ineffective, surgery may be recommended.

General Measures: Exercise is recommended and social activities are encouraged. However, people who have a seizure disorder may have to make some adjustments. For example, they should eliminate or limit their consumption of alcoholic beverages and should not use recreational drugs. They should refrain from activities in which a sudden loss of consciousness could result in serious injury. For example, they should not bathe in a bathtub, climb, swim, or operate power tools. After seizures are controlled (typically for at least 6 months), they can do these activities if adequate precautions are taken. For example, they should swim only when lifeguards are present. In most states, laws prohibit

people with a seizure disorder from driving until they have been free of seizures for at least 6 months to 1 year.

A family member or close friend should be trained to help if a seizure occurs. Attempting to put an object (such as a spoon) in the person's mouth to protect the person's tongue should not be tried. Such efforts can do more harm than good. The teeth may be damaged, or the person may bite the helper unintentionally as the jaw muscles contract. However, helpers should do the following during a seizure:

- Protect the person from falling
- Loosen clothing around the neck
- Place a pillow under the head

If a pillow is unavailable, helpers can put their foot or place an item of clothing under the person's head.

People who lose consciousness should be rolled onto one side to ease breathing. People who have had a seizure should not be left alone until they have awakened completely, are no longer confused, and can move about normally. Usually, their doctor should be notified.

Anticonvulsants: These drugs reduce the risk of having another seizure. Usually, they are prescribed only for people who have had more than one seizure, unless the cause has been identified and completely eliminated. They are usually not prescribed when people have had only one generalized seizure. Most anticonvulsants are taken by mouth.

Anticonvulsants can completely prevent generalized seizures in about one third of people who have them and greatly reduce the frequency of seizures in another third. Almost two thirds of people who respond to anticonvulsants can eventually stop taking them without having a relapse. However, anticonvulsants are ineffective in about 10 to 20% of people with a seizure disorder. These people are referred to a seizure center and evaluated for surgery.

There are many different types of anticonvulsants. Which one is effective depends on the type of seizure and the response to it. For most people, taking one anticonvulsant, usually the first or second one tried, controls seizures. If seizures recur, different anticonvulsants are tried. Determining which anticonvulsant is effective may take several months. Some people have to take several drugs, which increases the risk of side effects. Some anticonvulsants are not used alone but only with other anticonvulsants.

Doctors take care to determine the appropriate dose of an anticonvulsant for each person. The best dose is the smallest dose that stops all seizures while having the fewest side effects. Doctors ask people about side effects, and then adjust the dose if needed. Sometimes doctors also measure the level of anticonvulsant in the blood. Anticonvulsants should be taken just as prescribed. People who take anticonvulsants to control seizures should see a doctor regularly for dose adjustment and should always wear a Medic Alert bracelet inscribed with the type of seizure disorder and the drug being taken.

Anticonvulsants can interfere with the effectiveness of other drugs, and vice versa. Consequently, people should make sure their doctor knows all the drugs they are taking before they start taking anticonvulsants. They should also talk to their doctor and possibly

their pharmacist before they start taking any other drugs, including over-the-counter drugs.

After seizures are controlled, people take the anticonvulsant until they have been seizure-free for at least 2 years. Then, the dose of the drug may be decreased gradually, and the drug eventually stopped. If a seizure recurs after the anticonvulsant is stopped, people may have to take an anticonvulsant indefinitely. Seizures usually recur within 2 years if they are going to. A recurrence is more likely in people who have had any of the following:

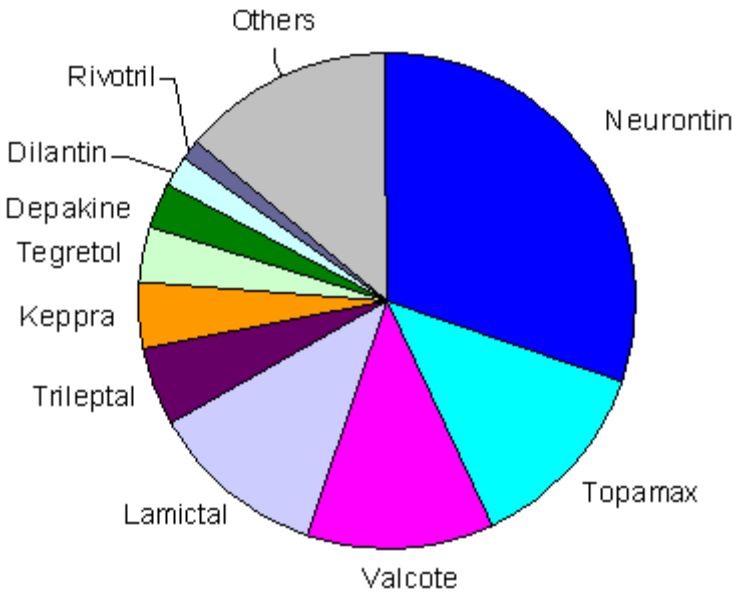
- A seizure disorder since childhood
- The need to take more than one anticonvulsant to be seizure-free
- Seizures while taking an anticonvulsant
- Partial or myoclonic seizures
- Abnormal EEG results within the previous year

Anticonvulsants, although very effective, may have side effects. Many cause drowsiness, but some may make children hyperactive. Blood tests are done periodically to determine whether an anticonvulsant is impairing kidney or liver function or reducing the number of blood cells. People taking anticonvulsants should be aware of possible side effects and should consult their doctor at the first sign of side effects.

For women who have a seizure disorder and are pregnant, taking an anticonvulsant increases the risk of miscarrying or of having a baby with a birth defect.

However, stopping the anticonvulsant may be more harmful to the woman and the baby. Having a generalized seizure during pregnancy can injure or kill the fetus. All women who are of childbearing age and take an anticonvulsant should take folate supplements to reduce the risk of having a baby with a birth defect.

Emergency Treatment: Emergency treatment is required for status epilepticus and seizures that last more than 5 minutes. Large doses of one or more anticonvulsants are given intravenously as quickly as possible. Measures to prevent injuries are taken during the prolonged seizure. People are monitored closely to make sure breathing is adequate. If it is not, a tube is inserted to help with breathing—a procedure called intubation. If seizures persist, a general anesthetic is given to stop them.



Present global anti-epileptic market[7]

2.7 Marketed Drugs Used to Treat Seizures[7]

Table - I

Brand Name	Drug	Use
Diamox	Acetazolamide	Absence seizures when other anticonvulsants are ineffective.
Tegretol	Carbamazepine	Effective against generalised tonic clonic and partial seizures. Ineffective against absences.
Frisium: Tablets 10mg	Clobazam	Effective against generalised tonic clonic and partial seizures, but tolerance develops in about one third of children
Rivotril: Tablets 0.5mg, 2mg.	Clonazepam	Effective against generalised tonic clonic and partial seizures, absences, myoclonic seizures, Lennox-Gastaut syndrome, infantile spasms and status epilepticus.
Emeside: Syrup 250mg/5mL. Zarontin: Syrup 250mg/5mL.	Ethosuximide	Effective against generalised absences. May be used for epilepsies with similar EEG changes to absences
Neurontin:	Gabapentin	Recommended in partial seizures where

Tablets 600mg, 800mg. Capsules 100mg, 300mg,		previous treatment has been ineffective.
Lamictal: Tablets 25mg, 50mg, 100mg, 200mg. Dispersible tablets 2mg 5mg, 25mg, 100mg	Lamotrigine	Effective against partial, absence, generalised tonic clonic seizures and Lennox-Gastaut syndrome.
Keppra: Tablets 250mg, 500mg, 1000mg. Oral solution 100mg/ml.	Levetiracetam	Can be used in children with partial or generalised seizures, over 4 years of age.
Suspension 2.5mg/5ml	Nitrazepam	Infantile spasms.
Trileptal: Tablets 150mg, 300mg,	Oxcarbazepine	Partial and generalised seizures.
Epanutin: Capsules 25mg, 50mg, 100mg, 300mg. Chewable Infatabs 50mg. Suspension 30mg/5mL.	Phenytoin	Effective against generalised tonic clonic and partial seizures. Status epilepticus. Blood testing is essential when using phenytoin as the relationship between dose and blood level is complex.
Mysoline: Tablets 250mg.	Primidone	Partial and generalised tonic clonic seizures.

Sabril: Tablets 500mg. Powder sugar free 500mg per sachet.	Vigabatrin	Can be considered for resistant partial seizures if visual fields can be monitored. First line for infantile spasms. May worsen absences and myoclonic seizures.
Topamax: Tablets 25mg, 50mg, 100mg, 200mg. Sprinkle capsules 15mg, 25mg, 50mg.	Topiramate	Recommended in partial and generalised seizures. Severe myoclonic epilepsy in infancy. For children over 2 years old.
Gabitril: Tablets 5mg, 10mg, 15mg	Tiagabine	Recommended in partial seizures when previous treatment has been ineffective. May make myoclonic seizures worse
Epilim: Tablets 200mg, 500mg. Crushable tablets 100mg. Liquid sugar free 200mg/5mL. Syrup 200mg/5mL. Epilim Chrono: Tablets 200mg, 300mg, 500mg.	Sodium valproate	Effective against generalised tonic clonic and partial seizures and absences.

3.0 OBJECTIVES OF THE STUDY

The following are objectives of the study

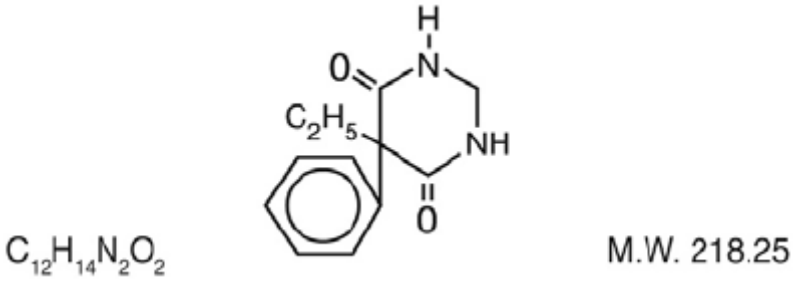
- To assess the bioequivalence of the test product 'T' and compare with the reference product 'R' in 14 normal healthy male adult human subjects under fasting conditions.
- To monitor adverse events and ensure the safety of subjects.
- To investigate the source of observed variability in the C_{max} of the test drug.

4. DRUG REVIEW[8]

4.1 Description

Chemical name: 5-ethyldihydro-5-phenyl-4, 6 (1H, 5H) pyrimidinedione.

Structural formula:



Primidone is a white, crystalline, highly stable substance, M.P. 279-284°C. It is poorly soluble in water (60 mg per 100 mL at 37°C) and in most organic solvents. It possesses no acidic properties, in contrast to its barbiturate analog.

Each tablet, for oral administration, contains 50 mg Primidone. In addition, each tablet contains the following inactive ingredients: colloidal silicon dioxide, ocusate sodium, magnesium stearate, microcrystalline cellulose, sodium benzoate, sodium starch glycolate and stearic acid.

4.2 Pharmacokinetic profile

Absorption

Readily absorbs from the GI tract. T_{max} is 3 h (Primidone) and 7 to 8 h (Phenobarbital and phenylethylmalonamide [PEMA] metabolites). Bioavailability is 90% to 100%.

Distribution

Protein binding is negligible for Primidone and PEMA; approximately 50% (Phenobarbital). Distributes in to breast milk. Vd is 0.64 to 0.86 L/kg.

Metabolism

Primidone converts to Phenobarbital and phenylethylmalonamide (PEMA). PEMA is the major metabolite and is less active than Phenobarbital. It is still unknown which exact cytochrome P450 enzymes are responsible. The rate of Primidone metabolism was greatly accelerated by Phenobarbital pretreatment, moderately accelerated by Primidone pretreatment, and reduced by PEMA pretreatment. The rate of metabolism of Primidone into Phenobarbital was inversely related to age; the highest rates were in the oldest patients (the maximum age being 55).

Elimination

Excreted in the urine (40% as unchanged and remainder excreted as metabolites). Plasma $t_{½}$ is 5 to 15 h (Primidone), 10 to 18 h (PEMA), and 53 to 140 h (Phenobarbital).

4.3 Pharmacodynamic profile[9]

Primidone is a barbiturate with anticonvulsant properties. Primidone, either alone or used concomitantly with other anticonvulsants, is indicated in the control of grand mal, psychomotor, and focal epileptic seizures. It may control grand mal seizures refractory to other anticonvulsant therapy. Primidone raises electro- or chemoshock seizure thresholds or alters seizure patterns in experimental animals. Primidone per se has anticonvulsant activity as do its two metabolites, Phenobarbital and phenylethylmalonamide (PEMA). In addition to its anticonvulsant activity, Primidone potentiates that of Phenobarbital in experimental animals.

Primidone is a GABA receptor agonist. The mechanism of **PRIMIDONE** antiepileptic action is exactly not known.

Indications and Usage

Primidone indicated in the control of grand mal, psychomotor, and focal epileptic seizures. It may control grand mal seizures refractory to other anticonvulsant therapy.

Adverse Events

The most common events observed in clinical trials with Primidone that ataxia, vertigo, nausea, anorexia, vomiting, fatigue, hyperirritability, emotional disturbances, diplopia, nystagmus, and drowsiness.

4.4 Drug Interaction profile

Induces CYP1A2 (strong), 2B6 (strong), 2C8/9 (strong), 3A4 (strong)

Acetaminophen: Primidone may enhance the hepatotoxic potential of acetaminophen overdoses.

Antiarrhythmics: Primidone may increase the metabolism of antiarrhythmics, decreasing their clinical effect; includes disopyramide, propafenone, and quinidine.

Anticonvulsants: Primidone may increase the metabolism of anticonvulsants; includes ethosuximide, felbamate (possibly), lamotrigine, phenytoin, tiagabine, topiramate, and zonisamide; does not appear to affect gabapentin or levetiracetam.

Antineoplastics: Limited evidence suggests that enzyme-inducing anticonvulsant therapy may reduce the effectiveness of some chemotherapy regimens (specifically in ALL); teniposide and methotrexate may be cleared more rapidly in these patients.

Antipsychotics: Primidone may enhance the metabolism (decrease the efficacy) of antipsychotics; monitor for altered response; dose adjustment may be needed.

Beta-blockers: Metabolism of beta-blockers may be increased and clinical effect decreased; atenolol and nadolol are unlikely to interact given their renal elimination.

Calcium channel blockers: Primidone may enhance the metabolism of calcium channel blockers, decreasing their clinical effect.

Chloramphenicol: Primidone may increase the metabolism of chloramphenicol and chloramphenicol may inhibit barbiturate metabolism; monitor for altered response.

Cimetidine: Primidone may enhance the metabolism of cimetidine, decreasing its clinical effect.

CNS depressants: Sedative effects and/or respiratory depression with Primidone may be additive with other CNS depressants; monitor for increased effect. Includes ethanol, sedatives, antidepressants, narcotic analgesics, and benzodiazepines.

Corticosteroids: Primidone may enhance the metabolism of corticosteroids, decreasing their clinical effect.

Cyclosporine: Levels may be decreased by Primidone.

CYP1A2 substrates: Primidone may decrease the levels/effects of CYP1A2 substrates. Example substrates include aminophylline, estrogens, fluvoxamine, mirtazapine, ropinirole, and theophylline..

CYP2B6 substrates Primidone may decrease the levels/effects of CYP2B6 substrates. Example substrates include bupropion, efavirenz, promethazine, selegiline, and sertraline.

CYP2C8/9 substrates: PRIMIDONE may decrease the levels/effects of CYP2C8/9 substrates. Example substrates include amiodarone, fluoxetine, glimepiride, glipizide, losartan, nateglinide, phenytoin, pioglitazone, rosiglitazone, sertraline, sulfonamides, warfarin, and zafirlukast.

CYP3A4 substrates: PRIMIDONE may decrease the levels/effects of CYP3A4 substrates. Example substrates include benzodiazepines, calcium channel blockers, clarithromycin, cyclosporine, erythromycin, estrogens, mirtazapine, nateglinide, nefazodone, nevirapine, protease inhibitors, tacrolimus, and venlafaxine.

Doprimidonecycline: **PRIMIDONE** may enhance the metabolism of doprimidonecycline, decreasing its clinical effect; higher dosages may be required

Estrogens: PRIMIDONE may increase the metabolism of estrogens and reduce their efficacy.

Felbamate may inhibit the metabolism of **PRIMIDONE** and **PRIMIDONE** may increase the metabolism of felbamate.

Griseofulvin: PRIMIDONE may impair the absorption of griseofulvin, and griseofulvin metabolism may be increased by **PRIMIDONE**, decreasing clinical effect.

Guanfacine: Effect may be decreased by **PRIMIDONE.**

Immunosuppressants: PRIMIDONE may enhance the metabolism of immunosuppressants, decreasing its clinical effect; includes both cyclosporine and tacrolimus.

Loop diuretics: Metabolism may be increased and clinical effects decreased; established for furosemide, effect with other loop diuretics not established.

MAO inhibitors: Metabolism of **PRIMIDONE** may be inhibited, increasing clinical effect or toxicity of the **PRIMIDONE.**

Methadone: PRIMIDONE may enhance the metabolism of methadone resulting in methadone withdrawal.

Methoprimidoneflurane: PRIMIDONE may enhance the nephrotoxic effects of methoprimidoneflurane.

Oral contraceptives: **PRIMIDONE** may enhance the metabolism of oral contraceptives, decreasing their clinical effect; an alternative method of contraception should be considered.

Theophylline: PRIMIDONE may increase metabolism of theophylline derivatives and decrease their clinical effect.

Tricyclic antidepressants: PRIMIDONE may increase metabolism of tricyclic antidepressants and decrease their clinical effect; sedative effects may be additive.

Valproic acid: Metabolism of **PRIMIDONE** may be inhibited by valproic acid; monitor for excessive sedation; a dose reduction may be needed.

Warfarin: PRIMIDONE inhibit the hypoprothrombinemic effects of oral anticoagulants via increased metabolism; this combination should generally be avoided.

Contraindications:

PRIMIDONE is contraindicated in:

1) Patients with porphyria

2) Patients with clinically significant hypersensitivity to the drug or any of the components contained in the formulation.

4.5 Therapeutic Dosage

Therapeutic dose of **PRIMIDONE** ranges from 50 mg to 250 mg depending upon the indication and the severity of underlying condition.

Precautions

The abrupt withdrawal of antiepileptic medication may precipitate status epilepticus.

The therapeutic efficacy of a dosage regimen takes several weeks before it can be assessed.

Usage in Pregnancy

The effects of **PRIMIDONE** in human pregnancy and nursing infants are unknown. Recent reports suggest an association between the use of anticonvulsant drugs by women with epilepsy and an elevated incidence of birth defects in children born to these women. Data are more extensive with respect to diphenylhydantoin and Phenobarbital, but these are also the most commonly prescribed anticonvulsants; less systematic or anecdotal reports suggest a possible similar association with the use of all known anticonvulsant drugs.

The reports suggesting an elevated incidence of birth defects in children of drug treated epileptic women cannot be regarded as adequate to prove a definite cause and effect relationship.

There are intrinsic methodologic problems in obtaining adequate data on drug teratogenicity in humans; the possibility also exists that other factors leading to birth defects, e.g., genetic factors or the epileptic condition itself may be more important than drug therapy. The great majority of mothers on anticonvulsant medication deliver normal infants.

It is important to note that anticonvulsant drugs should not be discontinued in patients in whom the drug is administered to prevent major seizures because of the strong possibility of precipitating status epilepticus with attendant hypoxia and threat to life. In individual cases where the severity and frequency of the seizure disorders are such that the removal of medication does not pose a serious threat to the patient, discontinuation of the drug may be considered prior to and during pregnancy, although it cannot be said with any confidence that even minor seizures do not pose some hazard to the developing embryo or fetus.

The prescribing physician will wish to weigh these considerations in treating or counseling epileptic women of childbearing potential.

Neonatal hemorrhage, with a coagulation defect resembling vitamin K deficiency, has been described in newborns whose mothers were taking **PRIMIDONE** and other anticonvulsants. Pregnant women under anticonvulsant therapy should receive prophylactic vitamin K_1 therapy for one month prior to, and during, delivery.

The total daily dosage should not exceed 2 g. Since **PRIMIDONE** therapy generally extends over prolonged periods, a complete blood count and a sequential multiple analysis-12 (SMA-12) test should be made every six months.

In Nursing Mothers

There is evidence that in mothers treated with **PRIMIDONE**, the drug appears in the milk in substantial quantities. Since tests for the presence of **PRIMIDONE** in biological fluids are too complex to be carried out in the average clinical laboratory, it is suggested that the presence of undue somnolence and drowsiness in nursing newborns of **PRIMIDONE** treated mothers be taken as an indication that nursing should be discontinued.

5. Literature review

1) Borst SI, et al.,(1975)[10]Comparative plasma level studies on different brands of sodium diphenylhydantoin (DPH) and primidone are described. Steady state plasma levels of both drugs were measured in epileptic patients who were chronically maintained on this medication. Simultaneous measurements of phenobarbital, primidone and diphenylhydantoin were carried out by gas chromatography. Drug product equivalence and clinical significance of plasma levels of DPH and primidone are discussed.

2) Meyer MC at al., (1993) [11] Problems related to bioequivalence and bioavailability for four antiepileptic drugs (AEDs) are reviewed. Bioequivalence and bioavailability of AEDs can be affected by many factors, including physicochemical characteristics of the agent, the dosage form, and physiological condition of the patient. In 1988, breakthrough seizures prompted an FDA investigation of one company's generic carbamazepine tablets. Results indicated that the manufacturer had changed its source of carbamazepine, which led to a wide range of dissolution characteristics for different lots of tablets. In two separate studies, clonazepam was shown to be more rapidly absorbed in patients with a normal gastric pH than in those with a higher-than-normal gastric pH. With phenytoin, which exhibits nonlinear pharmacokinetics, differences in the rate and extent of absorption can adversely affect the bioavailability of this agent. Finally, the bioequivalence of generic primidone was contested in an adolescent girl who appeared to experience more frequent seizures with a generic product than with a trade formulation. The effectiveness of a drug depends on complex interactions

involving the drug, the drug product formulation, and the patient. Minimizing variability in the absorption process is particularly important with AEDs, because of their narrow therapeutic range.

3) Agarwal S et al., (12)A randomized, two-way crossover study was conducted in 24 fasting healthy male volunteers of Indian origin to compare the bioavailability of two brands of a fixed dose combination of escitalopram oxalate (CAS 219861-08-2) 10 mg and clonazepam (CAS 1622-61-3) 0.5 mg tablets, using Estomine-zee as test and a commercially available formulation as the reference product. The pharmacokinetics of escitalopram oxalate and clonazepam individually after oral administration of tablet formulation has been extensively evaluated in adult volunteers. However, no published data are available regarding the pharmacokinetics and bioavailability of this particular fixed dose combination. METHOD: The trial was designed as a randomized, balanced, open-label, 2-period cross-over study. The drug was administered with 240 ml of water after a 10-h overnight fasting on two treatment days separated by a 21-day washout period. After dosing, serial blood samples were collected for a period of 96 h. Plasma harvested from blood was analyzed by simple rapid, selective and validated liquid chromatography-electrospray mass spectrometry (LC-ESI-MS/ MS) using diazepam (CAS 439-14-5) as an internal standard. RESULTS: The calibration curves were found to be linear in the range of 1-25 ng/ml and 1-10 ng/ml for escitalopram oxalate and clonazepam, respectively, with a mean correlation coefficient of more than 0.99. No statistically significant differences were

obtained between the two products with respect to the mean concentration-time profiles or in the pharmacokinetic parameters, including the area under the serum concentration-time curve from the present study. CONCLUSION: Based on the statistical inferences, it was concluded that the test product is bioequivalent to the reference product. Both preparations were well tolerated with no adverse reactions throughout the study.

4) Kongpatanakul S, et al.,(13)Oseltamivir, an ester prodrug of its active carboxylate metabolite, is an effective neuraminidase inhibitor used to treat influenza A and B virus infections. The purpose of this study was to compare the bioavailability of two 75 mg oral formulations of oseltamivir: a generic drug, GOP-A-Flu (test, Government Pharmaceutical Organization, Thailand) and Tamiflu (reference, Hoffmann-La Roche Ltd., Nutley, NJ, USA) in healthy volunteers. SUBJECTS AND METHODS: A single-dose, randomized, 2-sequence, crossover study was conducted in 24 healthy Thai volunteers. Each volunteer received a 75 mg capsule of the reference or test drugs under fasting conditions. Blood samples were collected before dosing and at various time points up to 48 hours after dosing and analyzed for plasma oseltamivir and oseltamivir carboxylate concentrations. The pharmacokinetic parameters including Cmax, AUC0-t, AUC0-infinity, tmax and t1/2 were analyzed using the non-compartmental method. Drug safety was assessed. RESULTS: 23 volunteers completed both treatment periods. The geometric mean ratios (test/reference) between the two formulations of oseltamivir were

96.83% (90% CI, 76.85 - 123.15%) for Cmax 103.66% (86.44 - 113.56%) for AUC0-t, and 103.98% (86.44 - 113.56%) for AUC0-infinity. Those of oseltamivir carboxylate were 102.17% (90% CI, 90.90 - 109.10%) for Cmax, 103.95% (90.90 - 109.10%) for AUC0-t, and 103.95% (90.92 - 109.08%) for AUC0-infinity. No significant difference of the tmax of oseltamivir and oseltamivir carboxylate between the two formulations was detected ($p > 0.05$). Both formulations were well-tolerated. CONCLUSION: Although the Cmax of oseltamivir was the only parameter not entirely within the equivalence criteria, the two capsule formulations were considered bioequivalent in terms of rate and extent of absorption regarding its active carboxylate metabolite.

5) Henney HR 3rd et al., (14) BACKGROUND: The alpha2-adrenergic agonist tizanidine has been reported to have a narrow therapeutic index. A multiparticulate capsule formulation of tizanidine has been developed in an attempt to improve patient tolerability. OBJECTIVE: This study assessed bioequivalence between a single, intact, 6-mg capsule of tizanidine and the capsule contents sprinkled in applesauce in fasted healthy subjects. METHODS: Healthy male and female subjects aged 18 to 45 years completed 2 treatment periods: one with a tizanidine 6-mg capsule administered intact and the other with capsule contents sprinkled in applesauce. The 2 treatment periods had a 6-day washout period between administrations. Plasma tizanidine concentrations were determined for blood samples collected over 24 hours after administration. All treatment-emergent adverse events were

recorded and graded by intensity and relationship to the study drug (not, improbable, possible, probable, definite) by the attending physician based on his or her clinical impression. RESULTS: A total of 19 men and 9 women (mean age, 26 years) completed the trial. Geometric mean natural logarithm-transformed AUC values (AUC(0-infinity) [AUC to infinity] and AUC(0-t) [AUC to the last measurable time point]) and C(max) ratios were significantly (P <or= 0.035) increased to 1.14 (90% CI, 105.47%-127.01%), 1.16 (90% CI, 106.80%-130.53%), and 1.17 (90% CI, 103.95%-133.66%), respectively, when the contents were sprinkled, with 90% CIs laying outside the 0.80 to 1.25 ratio established by regulatory authorities for bioequivalence. A total of 31 adverse events were reported by 17 of the 28 subjects (61%), including 15 subjects (54%) with the intact capsule reporting 18 events and 11 subjects (39%) with the sprinkled contents reporting 13 events. No serious adverse events or deaths were reported, and no subjects were discontinued due to adverse events. CONCLUSIONS: The contents of the tizanidine capsule sprinkled in applesauce were not bioequivalent to the intact 6-mg capsule in these fasted healthy volunteers. Therefore, if switching from the intact capsule to the capsule contents mixed in applesauce, monitoring for adverse events is recommended; in this situation, dose adjustment could be necessary.

6.0 DESIGN AND CONDUCT OF STUDY[15]

A bioequivalence study is basically a comparative bioavailability study designed to establish equivalence between test and reference products. The design should be based

on a reasonable knowledge of the pharmacokinetics of the active substance in question. The design and conduct of the study should follow ICH/ EU-regulations on Good Clinical Practice, including reference to an Ethics Committee. The rights, safety, and well being of all trial subjects must always be respected and should be given special attention.

DESIGN OF BA/BE FACILITIES

A general BE study facility includes various departments like the Clinical department, Bioanalytical department, Bio-statistics and Data management division and a Quality assurance (QA) department. Each department in turn consists of different functional areas.

The Clinical facility has many subdivisions

- Clinical Pharmacological Units (CPU) with areas for Phlebotomy, Dosing stations, recreation and refreshment rooms with safety precautions taken.
- Separate areas for, sample separation, deep freezers.
- Full time medical vigilance and care.
- ICU and emergency medical care services.
- Diagnostic lab services.
- Biowaste disposal services.

The Bioanalytical facility comprises of various functional areas like:

- Analytical lab.
- Sample processing room.
- Washing room.
- Store room for chemicals and solvents.
- Mass balance room.

The pharmacokinetics and statistics division is associated with functions like:

- Pharmacy.
- Sample size calculations.
- Statistical analysis.
- Study design.

- Data collection, verification and analysis.
- Report preparation as per the regulatory standards.

Quality Assurance department generally functions as an independent unit for indirect enforcement of stringent quality standards to the whole study process and system. It is responsible for planning and conducting regular audits in all the departments to ensure that all the activities are carried out in accordance to the approved protocol, and in accordance with the laboratory standards. It independently verifies all the raw data generated during the study process for its completeness, accuracy and authenticity.

6.1 STUDY DESIGN

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If the number of formulations to be compared is two, a two-period, two-sequence crossover design is often considered to be the design of choice. However, under certain circumstances and provided the study design and the statistical analyses are scientifically sound alternative well-established designs could be considered such as parallel design for very long half-life substances and replicate designs for substances with highly variable disposition

In the present study the design followed was open-label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, crossover bioequivalence study.

It is an open labeled study as the subjects and the investigator were not is blinded towards the identity of the study medications.

6.2 WASHOUT PERIOD:

Subsequent treatments should be separated by periods long enough to eliminate the previous dose before the next one (adequate wash out periods). In steady-state studies wash out of the previous treatment last dose can overlap with the build-up of the second

treatment, provided the build-up period is sufficiently long (at least three times the terminal half-life).

In the present study drug administration in first period was followed by a washout period of 14 days before subjects were switched over to the other treatment in the second period.

6.3 SELECTION OF SUBJECTS:

The subject population for bioequivalence studies should be selected with the aim to minimize variability and permit detection of differences between pharmaceutical products. Therefore, the studies should normally be performed with healthy volunteers. The inclusion/exclusion criteria should be clearly stated in the protocol. Subjects could belong to either sex; however, the risk to women of childbearing potential should be considered on an individual basis.

In general, subjects should be between 18 - 50 years old capable of giving informed consent and of weight within the normal range according to accepted normal values for the Body Mass Index (BMI) of 18-25. The BMI is calculated using the formula:

$$\text{BMI} = \frac{\text{Weight in Kgs}}{\text{Height in m}^2}$$

The number of subjects required is determined by

- The error variance associated with the primary characteristic to be studied as estimated from a pilot experiment, from previous studies or from published data,
- The significance level desired,
- The expected deviation from the reference product compatible with bioequivalence (delta, i.e. percentage difference from 100 %) and the required power.

The number of subjects required is calculated by the formula:

$$\mathbf{N= (t_{\alpha,\ 2N-2}+t_{\beta,\ 2N-2})^2\ [CV/\ (V-\delta)]^{\ 2}}$$

Where N=number of subjects

T=appropriate value from the t-distribution

α=type 1 error

β=type 2 error

δ=Treatment difference

CV=coefficient of variance (intra subject)

V=Bioequivalence limit

Calculations are quite tedious and time consuming, thus it is done using statistical software and statistical tools.

Table – II SUBJECT DEMOGRAPHICS[16]

S. No	Sub No	Smoking habit		Food habit		Age (years)	Height (cms)	Weight (kgs)	Remarks
		Yes	No	Veg	Non-Veg				

01	**01**	**--**	√	**--**	√	**22**	**162**	**53**	**--**
02	**02**	**--**	√	**--**	√	**28**	**168**	**70**	**--**
03	**03**	√	**--**	**--**	√	**20**	**166**	**64**	**1cigarette/day**
04	**04**	**--**	√	**--**	√	**21**	**157**	**50**	**--**
05	**05**	**--**	√	**--**	√	**22**	**160**	**57**	**--**
06	**06**	√	**--**	**--**	√	**28**	**169**	**74**	**1cigarette/day**
07	**07**	**--**	√	**--**	√	**22**	**166**	**54**	**--**
08	**08**	**--**	√	**--**	√	**20**	**165**	**53**	**--**
09	**09**	**--**	√	**--**	√	**21**	**166**	**53**	**--**
10	**10**	**--**	√	**--**	√	**22**	**168**	**56**	**--**
11	**11**	√	**--**	**--**	√	**20**	**165**	**62**	**2cigarettes/day**
12	**12**	**--**	√	**--**	√	**25**	**161**	**50**	**--**
13	**13**	**--**	√	**--**	√	**23**	**174**	**61**	**--**
14	**14**	**--**	√	**--**	√	**24**	**168**	**63**	**--**
AVERAGE						**22.7**	**165.3**	**58.5**	

STANDBY

Depending on the nature of adverse effects associated with the drug, there might be chances of subjects being dropped out from the study. In such cases, results obtained from the study might not be significant and unaccepted by him regulatory. To prevent such situation few subjects are included in the study as standby. The dropout subject data is replaced by another subject's data that was given the same sequence of treatment as the dropout during the study. Usually, 10% of the sample size is considered as standby.
Standby is decided on the basis of literature available about the drug and the pilot study. An even number of subjects on the higher side is taken as the number of subjects to be included in standby.

- In the present study, 14 subjects were included out of which 2 of them were standby. So the number of subjects participating in the study was represented as 12+2 standby.

6.4 PHARMACOKINETIC SAMPLING:

Under normal circumstances, blood, rather than urine or tissue, should be used. In most cases, drug, or metabolites are measured in serum or plasma. However, in certain cases whole blood may be more appropriate for analysis.

Blood samples should be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug. The sampling schedule should be planned to provide an adequate estimation of C_{max} and to cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of absorption. This is generally achieved if the AUC derived from measurements is at least 80% of the AUC extrapolated to infinity. For most drugs, 12 to 18 samples, including a predose sample, should be collected per subject per dose. This sampling should continue for at least three or more terminal half lives of the drug. The exact timing for sample collection depends on the nature of the drug and the input from the administered dosage form. The sample collection should be spaced in such a way that the maximum concentration of the drug in the blood (Cmax) and terminal elimination rate constant (λ_z) can be estimated accurately.

For drugs with a long half-life, relative bioavailability can be adequately estimated using condensed AUC as long as the total collection period is justified.
According to the C_{max} and T_{max} values of drug PRIMIDONE, the sampling schedule and amount of blood to be collected will be decided.

In each period, a total of 23 venous blood samples were collected from each subject as per the following schedule:

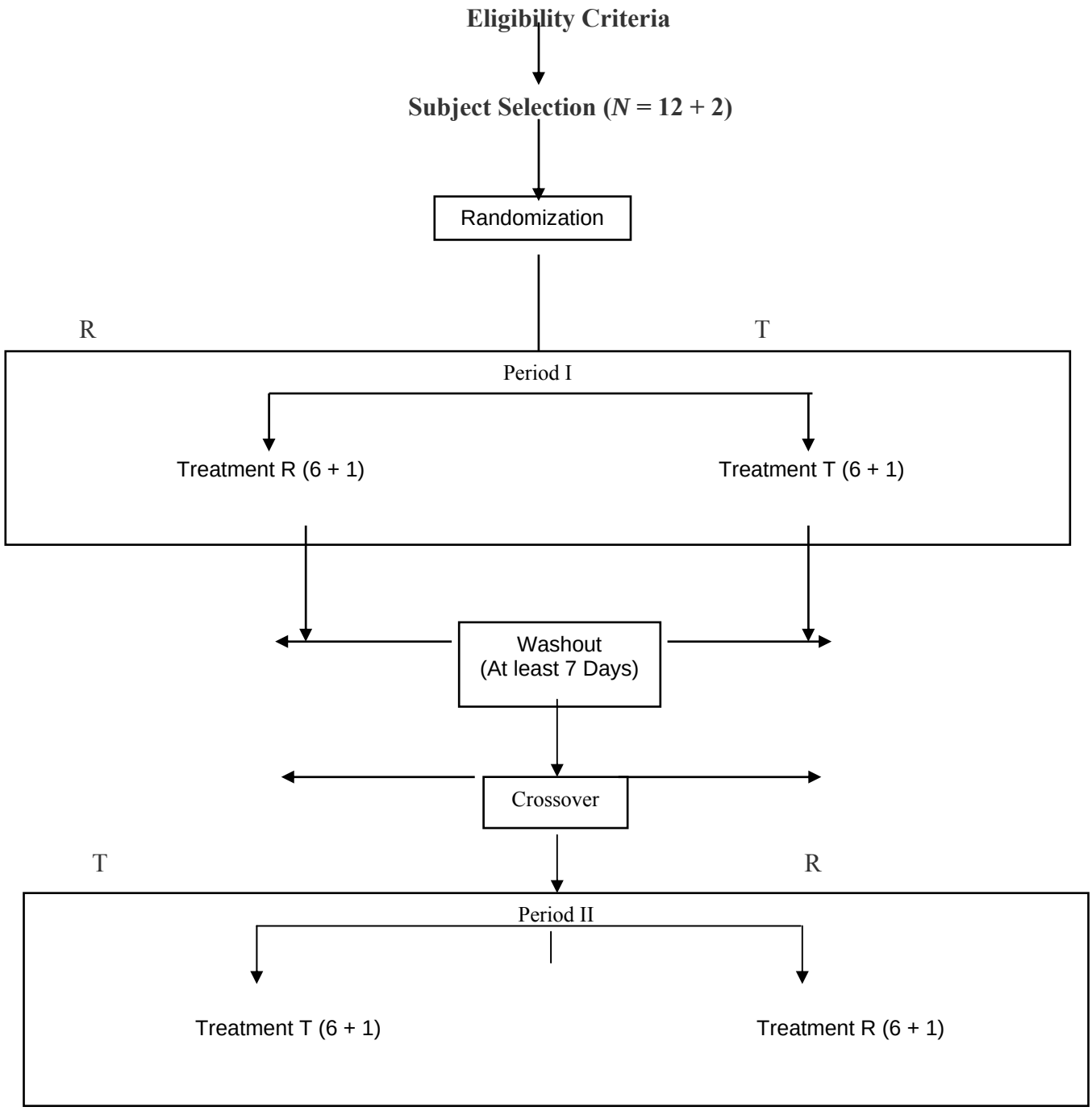
Predose (00 hr), 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post dose in each period.
The total volume of blood collected from each subject during the study did not exceed 241 ml. which included:

Table - III

Screening	10 ml
Study analysis	10 ml at the time of predose in each period.
In-house	203 ml(23*2*4ml+19ml discarded heparinized blood)
Post study	8 ml
Total	**241 ml**

STUDY FLOW CHART

Screening



6.5 PARAMETERS TO BE INVESTIGATED

In most cases evaluation of bioavailability and bioequivalence will be based upon the measured concentrations of the parent compound. In some situations, however, measurements of an active or inactive metabolite may be necessary instead of the parent

compound. The use of a metabolite may be advantageous to determine the extent of drug input, e.g. if the concentration of the active substance is too low to be accurately measured in the biological matrix (major difficulty in analytical method, product unstable in the biological matrix or half-life of the parent compound too short) thus giving rise to significant variability.

In bioavailability studies, the shape of the area under the plasma concentration versus time curves is mostly used to assess extent and rate of absorption. From the primary results, the bioavailability characteristics desired are estimated, namely AUC_{0-t}, $AUC_{0-\infty}$, Cmax, Tmax, as appropriate, or any other justifiable characteristics. In bioequivalence studies the AUC_{0-t} is the most reliable reflection of the extent of absorption.

The following pharmacokinetic parameters are required for submission:

- Plasma concentrations and time points.
- Subject, period, sequence, treatment.
- AUC_{0-t}, $AUC_{0-\infty,}$ Cmax, Tmax, λz, and $t_{1/2}$.
- Intersubject, intrasubject, and/or total variability, if available

The following statistical information required for AUC_{0-t}, $AUC_{0-\infty,}$ and Cmax:

- Geometric mean
- Arithmetic mean
- Ratio of means
- Confidence intervals

Rounding off of confidence interval values:

Confidence interval (CI) values should not be rounded off; therefore, to pass a CI limit of 80-125, the value should be at least 80.00 and not more than 125.00.

7.0 CLINICAL PHASE[17]

7.1 Communication from Sponsor

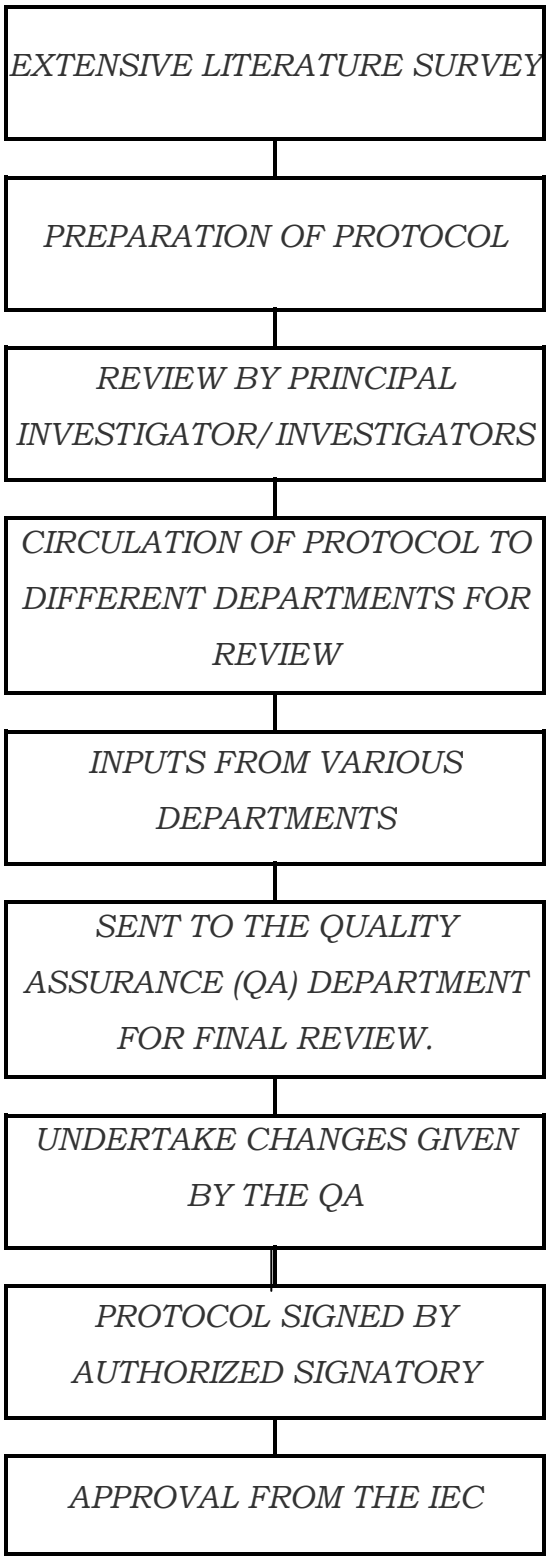
Sponsor is an individual, company, institution or organization which takes responsibility for the initiation, management, and financing of a clinical study for the Drug product under investigation.

The investigator of the study, after receiving information from the sponsor, starts preparing the protocol according to which the study is to be conducted.

7.2 Preparation of Protocol

Protocol is defined as a document signed and dated by the investigator and the sponsor that fully describes the objective(s), design, methodology, statistical considerations and organization of a study. The study protocol may also give the background and rationale for the study but these could be provided in other study protocol-referenced documents. The protocol includes all the details regarding the investigational product, the details regarding the administration of the drug, Pharmacokinetic (PK) sample withdrawal time-points, safety assessment parameters etc.

A protocol is prepared by the investigators of the study or his designee (usually a clinical pharmacologist and reviewed by various departments like analytical, statistical, QA to make necessary changes). The following chart gives an overview regarding the preparation of the protocol:


EXTENSIVE LITERATURE SURVEY
PREPARATION OF PROTOCOL
REVIEW BY PRINCIPAL INVESTIGATOR/ INVESTIGATORS
CIRCULATION OF PROTOCOL TO DIFFERENT DEPARTMENTS FOR REVIEW
INPUTS FROM VARIOUS DEPARTMENTS
SENT TO THE QUALITY ASSURANCE (QA) DEPARTMENT FOR FINAL REVIEW.
UNDERTAKE CHANGES GIVEN BY THE QA
PROTOCOL SIGNED BY AUTHORIZED SIGNATORY
APPROVAL FROM THE IEC

7.3 ETHICAL CONSIDERATIONS

Basic Principles

The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, Guideline for Good Clinical Practice and all Applicable regulations and guidance.

Institutional Review Board

An institutional review board / independent Ethics committee reviewed this protocol and the study started only after the approval of the protocol by the institutional review board / independent Ethics committee.

Independent Ethics Committee (IEC) consists of a board of members who look into the ethical issues of the study to be conducted. The study operations can be initiated only after the protocol is approved by IEC.

An IEC should safeguard the rights, safety, and well being of all trial subjects. Special attention should be paid to trials that may include vulnerable subjects. The investigator may provide information on any aspect of the trial, but should not participate in the deliberations of the IEC or in the vote/opinion of the IEC. An IEC may invite nonmembers with expertise in special areas for assistance

Composition, Functions, and Operations

The IEC consists of a reasonable number of members, who collectively have the qualifications and experience to review and evaluate the science, medical aspects, and ethics of the proposed trial. It is recommended that the IEC should include

(a) At least five members.

(b) At least one member whose primary area of interest is in a nonscientific area.

(c) At least one member who is independent of the institution/trial site.

- Only those IEC members who are independent of the investigator and the sponsor of the trial should vote/provide opinion on a trial-related matter.
- A list of IEC members and their qualifications should be maintained.
- It should perform its functions according to written operating procedures, should maintain written records of its activities and minutes of its meetings, and should comply with GCP and with the applicable regulatory requirement(s).
- Only members who participate in the IEC review and discussion should vote/provide their opinion and/or advice.

Informed Consent

Designated clinical research personnel informed the subjects before initiation of the study through an oral presentation regarding the purpose, procedures to be carried out, potential Hazards and rights of the subjects during the course of the study.

Termination of the study

The Study Can be terminated at any point of time at the interest of subjects welfare

7.4 Protocol Training

After the approval of the protocol, it is discussed among the investigators of the study. The summary of the protocol that includes:

- The name of the investigational product (drug to be administered to the subjects), Reference drug
- Dose to be administered
- Type of study whether it is a single center study, a fast or fed study, analyst study etc.
- Number of subjects to be enrolled in the study
- Kind of study etc, and other minute details like the Clinical Pharmacology unit (CPU) in which the subjects would be housed etc,

This summarized version of the protocol is discussed among the personnel in the facility to train them in the protocol.

7.5 Registration of Volunteers

For recruiting volunteers for a study suitable volunteers are selected from the database. New people are informed and registered in the database after they gave the written consent. Generally, healthy male, adult volunteers in the age group of 18-45 years are preferred according LIC height and weight chart.

- **Screening**

The screening will be carried out after taking an initial informed consent from volunteers for study screening procedures and will include the following:

1. Demographic data, including sex, height and weight, BMI, nutrition status, diet, history of smoking, substances of abuse (Benzodiazepines, opoids and Amphetamine), alcohol, blood donation and previous participation in drug research study.
2. Medical and treatment history including present complaints (if any), relevant past medical history, family history, history of any allergy to food or drug, medication history in the last six months.
3. Complete physical examination including recording of vital signs (B.P, Pulse, Temperature and respiratory rate) and systemic examination.
4. 12-lead ECG.
5. Chest X-ray (PA view)
6. Urine test to determine the consumption of drugs like morphine, codeine, LSD or any other drug of abuse (on the day of check in).
7. Blood and urine samples will not be sent for laboratory examination if the subject fails in eligibility assessment during medical examination.
8. Breathe alcohol test (on the day of check in).

- **Laboratory Parameter Investigations**

Table - IV

Pre–study laboratory evaluation parameters		
CLINICAL CHEMISTRY	**HEMATOLOGY**	**SEROLOGY**
Random blood sugar	Total W.B.C	HIV-1
Blood urea nitrogen(BUN)	Total R.B.C	HIV-2
creatinine	Hemoglobin	HbsAg
Total bilirubin	PCV	HCV
SGOT	Neutrophils	
SGPT	lymphocytes	
blood cholesterol	Mixed cells	
Total proteins	platelets	
Sodium	ESR(1hr)	
potassium		

- **Withdrawal Criteria**

Subjects may be withdrawn from the study by the principal investigator or co-investigators for any of the following reasons during the course of the study:

1. If the subject suffers from significant illness
2. If the subject requires concomitant medications which may interfere with pharmacokinetic of the study drug
3. If the subject has entered the study in violation of the inclusion and the exclusion criteria
4. If the subject is found to be non co-operative
5. If the subject decides to voluntarily dropout from the study

- **Note:**

Any such subject withdrawals will be reported for reasons for withdrawal (if any)

Medical examination of the subject will be done at the time of withdrawal / dropout,

The plasma concentration data from subjects who are withdrawn due to adverse events will be presented, but will not be included in the statistical analysis.

7.6 Subject Details

Total 14 healthy, adult, human, male volunteers were enrolled after drug of abuse screening and breath analysis for alcohol and all of them were admitted.

Gross Demographics Data

Average age of subjects in the study	: 22.7 years
Average weight of subjects in the study	: 58.5 kgs
Average height of subjects in the study	: 165.3 cms

Informed Consent Form (ICF)

ICF is designed as per the ICH-GCP and local regulatory requirements. ICF is conducted in order to get the consent from the volunteer to participate in the study. Volunteers were given all the information regarding the study including:

- Details of Investigational products
- Adverse events that may occur during the study
- The total blood loss
- The compensation to be given at the end of the study
- Regulations to be followed while participating in the study

Volunteers were given the freedom to withdraw from the study at any point of time, during the study. This consent is taken as a part of the ethical issue in conducting a BA/BE study. Every care was taken to protect the health of the volunteers. The volunteers signed on this form and gave their consent for participating in the study. Once they were enrolled in to the study, they were called '**subjects**'. The enrollment in the study started with the "**check-in**" process.

7.7Check-in Process

The volunteers who gave their consent to participate in the study were enrolled in the study i.e. the check-in process. During this process it is checked whether the person has met all the inclusion/exclusion criteria and cleared the screening process.

- **Inclusion Criteria**

1. Subjects who will provide written consent form.
2. Healthy males within the age range of 18 to 45 years
3. Preferably Non- Smokers
4. Willingness to provide written informed consent to participate in the study
5. Body mass index of ≥ 18.5 kg/m^2 and ≤ 24.9 kg/ m^2, with body weight not less than 50 kg
6. Absence of significant disease or clinically significant abnormal laboratory values or laboratory evaluation, medical history or physical examination during the screening
7. Have a normal 12-lead ECG or one with abnormality considered to be clinically insignificant
8. Have a normal chest X-ray.
9. Comprehension of the nature and purpose of the study and compliance with the requirement of the distributed ICF.

- **Exclusion Criteria**

1. Personal/Family history of allergy or hypersensitivity to the drug or allied drugs
2. Any major illness in the past 90 days or any clinically significant ongoing chronic medical illness e.g. Congestive Cardiac Failure (Heart failure), Hepatitis, Hypotensive episodes, Hyperglycemia etc
3. Presence of any clinically significant abnormal values during screening e.g. significant abnormality of liver function test, renal (kidney) function test etc
4. Severe cardiac, renal or liver impairment, gastro-intestinal disease or other conditions, any other organ or system impairment
5. History of seizures, epilepsy or any kind of Neurological disorders
6. Past history of Anaphylaxis or angioedema
7. Presence of disease markers of HIV – 1 and 2 and hepatitis B, C virus
8. Consumed alcohol within 48 hrs prior to dosing

9. Consumption of Xanthine containing derivatives (coffee, tea, cola – drinks, chocolate) or tobacco products within 48 hours prior to dosing
10. Use of any recreational drug or a history of drug addiction
11. Participation in any clinical trial within the past 90 days
12. History of difficulty with donating blood or difficulty in accessibility of veins in left or right arm
13. Donation of blood (one unit or 350 ml) within 90 days prior to receiving the first dose of study medication
14. Receipt of any other prescription drug or over the counter (OTC) drugs within two weeks prior to receiving the first dose of study medication or repeated use of drugs within the last four weeks
15. An unusual diet for whatever reason e.g. low sodium diet, for two weeks prior to receiving any medication and throughout subject's participation in the study
16. Recent history of dehydration from diarrhea, vomiting or any other reason within a period of 24 hours prior to the study

They will undergo vital examination and Medical examination again to ensure they are fit for participation in the study. They would be changing into the uniforms provided to them in the facility. Once the check-in of the volunteer is completed he would be called as 'subject'. The subjects are provided with all the requirements they need including recreational activities like movies and games, newspapers. The check in day is called as Day 0.The subjects are given standardized dinner after which they will be fasting overnight for 10 hours.

7.8 Randomization

The test and reference products were assigned to each subject in a sequence according to a predetermined randomization schedule prepared by using SAS software 9.1 version. The randomization schedule prepared is as follows:

Table - V

SUBJECT I.D	Sequence	Period - I	Period - II
001	1	T	R
002	2	R	T
003	1	T	R
004	2	R	T
005	2	R	T
006	1	T	R
007	1	T	R
008	2	R	T
009	1	T	R
010	2	R	T
011	2	R	T
012	1	T	R
013	1	T	R
014	2	R	T

7.9 Dispensing

As per the randomization schedule a qualified registered pharmacist dispensed the investigational products under the supervision of Quality Assurance personnel in both the periods. Remaining drug products were stored in their original container as retention samples. The test and reference product were stored in humidity chamber below 25°C and 60% RH ± 5%.

The dispensed tablets were transferred to the drug-dispensing containers as unit doses. The drug-dispensing containers used for dispensing were properly labeled for the

study number, period number, subject number, treatment code, initial and date of the person dispensing the product.

The details of the investigational project were as follows:

Table - VI

Test product T:	**Reference product R:**
PRIMIDONE tablets 50 mg	**PRIMIDONE** tablets 50 mg
Batch no: PDN-T050-F028	**Batch no: E0500110**
Description: White to off white, round, Flat, beveled edge, uncoated tablets, Embossed with RDY477on one side and break line on other side.	**Description:** White, square shaped, Flat, uncoated tablets embossed with 50 and break line on one side and "M" on other side of tablet.
Assay: Containing not less than 95.0% and not more than 105.0% of Test drug.	**Assay:** Containing not less than 99.40% and not more than 105.0%of Reference drug.

7.10 Dosing of Investigational product

The subjects were dosed next morning with the investigational product in the study after they have maintained 10hr fasting before dosing. The dosing day is called as Day 1. Dosing was done according to the procedure mentioned in the protocol and randomization code for the dosing is generated by the statistician, in which the sequence of investigational product administration was mentioned (eg. TR, RT). The investigational product was administered at

0.00 hours in presence of clinical investigator. It was observed that the subjects were administered with the Investigational product with fixed amount of water (240ml) and swallows it completely. Compliance for dosing after drug administration was assessed by examination of the oral cavity of the subjects by trained study personnel immediately after dose administration in each period.

Once the dosing was completed the blood samples from the subject were collected at time intervals mentioned in the protocol. One hour predose and 1 hours post dose water restriction was maintained.

- **DISTRIBUTION OF MEAL**

A standardized diet of approximately 2600 - 2800 calories per day was provided to the study subjects on the day of dosing.

On the day prior to dosing dinner will be provided. All subjects were served with standardized lunch, snacks, dinner at 4, 9, and 13 post-dose respectively.

7.11 Collection of blood samples

The subjects were cannulated on the day of dosing so that the blood withdrawal would be easier and to avoid the repeated needle pricks. Fixed volume of blood (generally 4 ml) was withdrawn at each time point. Samples were collected for all the subjects in each period pre-dose (00 hr), and 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post dose. This was collected in the vacutainers containing the adequate amount of anticoagulant as (generally K3 EDTA); to avoid the mixing of the blood with the residual blood in the cannula 0.5ml blood is discarded before every sample collection. After the sample is collected 0.5ml saline will be pushed in the cannula to avoid the blockage of the cannula. The collected blood samples are sent to the separation room where the plasma is separated. After centrifugation at defined parameters in the protocol (generally 3800 rpm at 10 ± 2 °C for 10 minutes) and the plasma samples were stored at - 20°C ± 10°C.

7.12 Safety monitoring

Safety assessment was carried out at the time of screening and during the course of study by conducting medical examination, recording vital signs and adverse event monitoring. Medical examination (including vital signs) was carried out in each period, at 0.00, 2.00, 4.00, 8.00, and 12.00 hours post dose.

The normal vital signs range is as follows:

Temperature	97.8 °F-99 °F
Pulse Rate	60-100 beats/min
Respiration Rate	14-20/min
Systolic BP	100-138mm of Hg
Diastolic BP	60-88mm of Hg

If any adverse event is observed either by clinical staff or reported by subjects at times other than scheduled times will also be recorded.

- **Handling and Reporting of Adverse Events**

During the course of the study, subjects were monitored for adverse event, which was recorded in the appropriate raw data forms.

The adverse events were recorded

- At the time of check-in for subsequent period.
- When reported by the subjects.
- At the time of the vitals measurement and subject well being verification.
- At the time of check out.

A medical officer was available round the clock during the time of housing at the clinical facility of Bioserve clinical Research Private Limited. All drug and/or study related adverse events would be treated by the medical officer free of cost either at clinic or at a suitable nearby hospital. Any adverse event discovered shall be appropriately treated and will be covered by an appropriate insurance policy. The ethics committee shall be informed immediately (within 24 hours) and regulatory bodies (whenever applicable)

shall also be informed of the serious adverse event as mandated by the rules and regulations.

Any serious adverse event will be reported to the Sponsor and Chairman of the Ethics Committee on identification either by telephone / fax within one working day of occurrence of event and full follow-up report to follow as soon as further information is available. Also, wherever applicable, regulatory authorities will be reported within 7 working days.

All adverse events shall be evaluated for duration, severity, action taken, date and time of resolution and causality with the study 'treatment'. The study may be suspended or terminated depending on the seriousness of adverse event.

Note: Adverse Drug Reactions (ADR) is all-noxious and unintended responses to a medicinal product related to any dose.

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product. This can include any unfavorable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests) or a disease temporarily associated with the use of the study medication.

A Serious Adverse Event (SAE) or reaction is any untoward medical occurrence that at any doses results in:

– Death

– Is life- threatening

Note: The term 'life- threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

– Requires inpatient hospitalization or prolongation of existing hospitalization

– Results in persistent or significant disability / incapacity, or

– Results in a congenital anomaly / birth defect.

The **expected** adverse events in the study were ataxia, vertigo, nausea, anorexia, vomiting, fatigue, hyperirritability, emotional disturbances, diplopia, nystagmus, and drowsiness.

The adverse events occurred in the study were as follows:

Table - VII

Subject Number	Period	Adverse Events	Action Taken
S_1	I	Drowsiness	Recovered without Medication
S_7	I	Vertigo	
S_{10}	I	Headache	
S_{11}	I	Vertigo	
S_{12}	I	Vertigo	
$S_{1, 3, 5, 8, 11,13}$	II	Drowsiness	

7.13 Check out process

After the completion of the study the subjects were checked- out. In the check out process the subjects undergo a medical check up to ensure that they are healthy even after participating in the study.

The study cycle was repeated after the washout period when the subjects are crossed over to other treatment.

Their post study medical check up includes the blood test. Once the subjects finish giving their blood samples they are paid their compensation.

7.14 Subject Compensation

The subjects will be compensated for the overall inconvenience borne during the study. In case of dropouts / withdrawal of a subject before completion of the study, the amount of proportionate compensation to the dropout / withdrawal subject will be as follows:

Table - VIII

Sr. No.	Reasons of Withdrawal from the Study	Compensation
1.	Principal Investigator / Medical Officer withdraw the subjects from the study based on medical decision.	Full payment
2.	After the initiation of the study, subject withdraws on his own free will	50% proportionate participation dues
3.	The subject is withdrawn from the study on humanitarian grounds, with the permission of the Principal Investigator / Medical Officer.	100% proportionate participation dues
4.	Subject is dropped from the study due to violation of requirements of the study by the Principal Investigator / Medical Officer after signing the Informed Consent Form but before receiving any medications	No payment
5.	Subject is withdrawn from the study by the Principal Investigator / Medical Officer because of willful misinformation on present and /or past medical	No payment

	illness/history.	

7.15 Sample separation and Storage

The collected blood samples received in vacutainers would be centrifuged at a particular RPM (3800 rpm) at a temperature of 10°C for around 10 minutes. The plasma gets separated due to the centrifugal force. This separated plasma is collected in two different tubes called aliquots. The aliquots are pre-labeled and sent to the deep freezer where the samples are stored at a temperature of –70 °C.

Various precautions are taken while separating the plasma. The plasma is separated without touching the bed of blood cells. Care should be taken that the samples are not exposed to room temperature for a long time.

7.16 Sample Sorting

Once all the samples from the subjects are collected, the samples are sorted. The sorting is done by separating the aliquots containing samples of different time points of each subject into easy sealing bags. Various conditions are maintained while sorting the samples, like the maintenance of low temperatures. Sorting is done in presence of dry ice to prevent the exposure of samples to room temperature and also to prevent their degradation due to thawing.

These bags are sealed into various boxes and stored in the deep freezer, which are later handed over to the bio-analytical department with proper documentation for further processing

7.17 Protocol Deviations

The details of protocol deviations occurred in the study are as follows:

Table - IX

Subject No	Sample Time point hr	Reason for deviation

S9	**48.0 hr**	**Absent**
S12	**12, 72 hr**	**Absent**

8.0 BIOANALYTICAL PHASE

The bioanalytical part of bioequivalence trials should be conducted according to the applicable principles of Good Laboratory Practice (GLP). (EMEA / OECD GLP / WHO GLP STANDARD). The bioanalytical methods used to determine the active moiety and/or its biotransformation product(s) in plasma, serum, blood or urine or any other suitable matrix must be well characterized, fully validated and documented to yield reliable results that can be satisfactorily interpreted.

8.1 Materials and Methods

The list of materials and methods used during analytical phase of the study are discussed below:

Method parameters

Table - X

Name Of the Drug	Primidone
Name Of the Analyte	Primidone
Molecular Wt. of Primidone	218.25
Molecular Formula of Primidone	$C_{12}H_{14}N_2O_2$
Name Of the Internal Standard	Oxcarbazepine

Molecular Wt. of Oxcarbazepine	252.27
Molecular Formula of Oxcarbazepine	$C_{15}H_{12}N_2O_2$
Calibration Range	20ng/mL to 2000 ng/ml
Lower Limit of Quantification	20ng/mL
Sample Processing Method	Liquid Liquid Extraction
Instrument Method	LC/MS/MS

Chemicals and Reagents

The following Chemicals were used in the estimation of Primidone

Table - XI

CHEMICAL	GRADE
Acetonitrile	HPLC
Methanol	HPLC
Ammonium Formate	Fluka
Formic Acid	GR/AR
Water	Milli Q
Tetra butyl methyl Ether	GR/AR
Sodium Hydroxide	GR/AR

8.2 Instrumentation

Shimadzu HPLC equipped with pump, auto sampler, Mass spectrometer **MDS SCIEX API 3200 LC/MS/MS** and data acquisition system **(Analyst Software Version 1.4.1)** were used for the quantitative determination of analyte in human plasma.

Chromatographic Conditions

LC conditions

Table - XII

Parameter	**Conditions**
Flow Rate	0.6 ml/min (Isocratic)
Injector Volume	40 µl
Auto sampler Temperature	5^0 C
Column Oven Temperature	40^0 C

MS/MS Conditions

Table - XIII

Parameter	**Primidone**	**Oxcarbazepine**

Declustering Potential	20	35
Entrance Potential	10	10
Collision Energy	35	40
Collision Cell Entrance Potential	20	25
Collision Cell Exit Potential	2	4
Polarity	Positive	Positive

8.3 Stock Solutions and sample Preparations

Diluents: Diluents were prepared by mixing Methanol and water in 1:1 ratio, mixed well and sonicated for 2 minutes.

Stock Solution Analyte (Primidone): 10.09 mg of Primidone reference standard was weighed and transferred into 10 ml of volumetric flask and added with 3 ml of methanol, sonicated to dissolve and made up to the mark with the same solvent.

Stock Solution Internal Standard (Oxcarbazepine): 10.05 mg of Oxcarbazepine reference standard is weighed and transferred into a 10 ml volumetric flask, 3ml of methanol is added and sonicated to dissolve and made up to the mark with the same solvent.

8.4 Preparation of Quality Control Samples

Quality control samples were Prepared using in selected human plasma as per the below model reference table. Quality control samples concentrations were calculated as per the actual concentration of the spiking solution, the spiking volume and the total volume were based on the requirement of the number of the quality control samples.

Table - XIV

Spiking Solutions		Diluent (ml)	Total Vol (ml)	Spiked solution Conc(ng/ml)	ID	Required Conc (ng/ml)	Ratio
CONC (µg/ml)	Vol (µl)						
75.0000	1.00	49.00	50.00	1500.0000	HQC	1500.0000	75% of highest
50.2500	1.00	49.00	50.00	1005.0000	MQC	1000.0000	50% of highest
2.7638	1.00	49.00	50.00	55.2760	LQC	55.0000	2.75 times of LLOQ
1.1055	0.2	9.80	10.00	22.1100	LLOQ QC	22.0000	110% of LLOQ

Storage of Matrix Samples

a) All matrix based samples were stored at -30^0 C or colder
b) Dry extract was stored at -10^0 or colder
c) Wet extracts were stored at 2 to 8^0 C

8.5 Sample Processing & Extraction Technique (LLE)

a) This is Applicable for the possessing of all matrix based samples
b) 500 µl of plasma was taken in a test tube and 50 µl of internal standard solution (400 nano gram per ml), vortex for 10 to 15 sec in a shaker.
c) 75 µl of 0.2 N NaOH is added to the test tube and vortex it for 15 to 20 seconds.
d) Add 3 ml of tetrabutyl methyl ether into the above test tubes and put in shaker under vibration for 10 min.
e) Centrifuge it for 5 minutes at 4000 rpm pipette out 2.7 ml of tetrabutyl methyl ether layer into another test tube and load into nitrogen evaporator
f) Evaporate the tetra butyl methyl ether to dryness at a temperature of 40^0 C and at a pressure of 5 psi initially for about 5 minutes, followed by 25 psi about 15 minutes
g) Add 250 µl of reconstitution solution (Mobile Phase), vortex for about 30 sec, transfer to auto injector vial and proceed for analysis.

8.6 Data Collection

All data collection and integration (area mode) were performed by statistical analysis software systems. The intercepts and goodness of fit were determined by least squares linear regression analyses using the ratios of drug / internal standard peak areas of the CC standards. The concentrations of all the study samples, CC standards and QC samples were calculated by Analyst version 1.4.1 software systems. A weighting factor of 1/ (concentration)^2 was used in the calculation of linear regression line with the following equation.

$$\mathbf{y = mx + b}$$

Where, y = peak area ratio of analyte / internal standard

m = slope of the CC

x = concentration ratio of analyte / internal standard

b = y-axis intercept of the C

Table - XV

The plasma concentrations (ng) of the drugs obtained were as follows

TIME POINTS	Subject-1		Subject-2		Subject-3		Subject-4	
	Period-1	Period-2	Period-1	Period-2	Period-1	Period-2	Period-1	Period-2
0.00	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
0.33	795.5122	947.2327	BLQ	168.6635	714.3559	BLQ	BLQ	30.3436
0.67	805.5066	823.9751	30.3668	723.3303	780.0156	260.5474	32.3929	407.6498
1.00	906.6731	922.9506	77.6182	804.4682	1083.7294	625.1278	70.6677	744.8823
1.33	885.5609	822.4703	102.3430	875.5967	1003.2728	901.6177	270.3452	602.6882
1.67	867.2002	910.5793	284.5106	886.0191	945.6885	1158.8768	654.6196	597.1412
2.00	919.2609	961.5539	306.0968	1142.7619	1114.7129	1268.5597	718.9361	590.6485
2.33	887.5220	838.3983	382..4266	875.9405	1068.7753	1161.0420	767.5626	799.6163
2.67	830.5983	938.2990	701.1376	864.9664	926.2478	1035.1097	736.001	603.7996
3.00	903.0058	847.0168	786.7926	1016.6159	1004.2299	1086.2361	757.4860	741.4913
3.33	800.9101	822.8384	870.8831	857.4108	941.5748	1003.2783	1197.8513	707.5560
3.67	828.3049	761.3667	847.2607	878.3686	953.2477	944.2588	800.6677	710.1647
4.00	855.5040	892.3737	1141.6796	896.0254	914.2805	1093.3308	572.1982	851.7415
5.00	787.6936	699.2319	867.7271	870.2898	887.0890	930.1700	666.8247	740.8939
6.00	770.330	794.6099	799.1474	853.6756	834.7647	948.7364	728.9634	824.2058
8.00	741.1016	710.4758	893.0873	829.5155	983.3764	748.6339	594.6363	675.5202

10.00	590.0876	639.2422	804.0446	647.3423	1131.7944	692.2361	586.9043	567.0021
12.00	598.7577	579.3651	756.6855	720.6503	661.9151	640.8641	610.9892	579.4316
16.00	485.4336	463.8485	674.6471	610.5195	557.1924	562.3615	433.0035	440.0556
24.00	408.3297	413.1775	595.8033	589.5981	454.6277	447.8442	314.3740	421.9569
36.00	188.9068	268.9414	333.3757	375.8187	227.6630	304.4049	254.0826	258.3301
48.00	158.2072	161.2934	190.3395	256.0387	156.8962	401.3480	186.1795	182.4546
72.00	63.5760	75.2760	93.5644	142.9080	77.8760	72.6972	84.6704	101.3456

TIME POINTS	**Subject-5**		**Subject-6**		**Subject-7**		**Subject-8**	
	Period-1	**Period-2**	**Period-1**	**Period-2**	**Period-1**	**Period-2**	**Period-1**	**Period-2**
0.00	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
0.33	366.6232	685.7622	338.5693	52.8629	284.4067	100.4543	488.3520	82.9419
0.67	578.2424	1185.8734	719.3155	722.8347	873.5804	710.8684	1086.5785	99.0105
1.00	912.2314	844.5620	1145.1738	621.7813	978.6609	744.2809	831.7903	217.5547
1.33	1024.3092	1072.4698	1067.8119	594.2504	713.1019	896.5370	710.8608	541.2518
1.67	1042.7022	1288.7934	1038.5385	1077.3324	1016.6410	758.5018	678.1115	906.5869
2.00	814.9772	1210.5706	1043.3136	1090.8598	954.3557	796. 2734	674.4253	709.9220
2.33	1035.8535	1277.7111	1012.6626	1162.8566	971.9115	836.9546	777.4437	717.6326
2.67	940.9772	1304.5853	1055.8744	1224.1304	966.5487	821.2462	642.3264	711.3024
3.00	968.4381	1233.4049	935.8594	1079.2374	1018.5572	930.7450	587.7479	688.9124
3.33	1240.5005	1244.5432	1022.3251	976.2727	935.7305	852.3651	688.0322	730.1991
3.67	1173.9863	1094.9792	1089.8835	1183.8235	925.1486	953.5073	596.8700	716.5353
4.00	1168.4691	1238.4336	1111.9109	1257.6883	702.9173	960.7402	643.3260	685.9113
5.00	1035.7931	1109.2431	1038.7816	1160.0757	769.0731	855.6945	499.5161	692.3572
6.00	1059.6896	1070.4614	952.3027	1097.8809	726.6649	845.8207	524.7505	742.2331
8.00	1013.3562	980.1173	938.7786	1051.2966	705.3668	718.1814	538.2990	690.3396
10.00	865.2928	910.8960	828.5804	1037.1813	634.0228	677.7919	542.4131	632.4632
12.00	884.8547	805.3492	807.9791	918.3238	580.6137	649.0068	514.8594	550.0999

16.00	609.2018	687.6082	584.1165	664.5009	452.5478	489.8529	460.7226	485.7548
24.00	655.3250	536.2725	505.3746	583.9011	373.8493	364.7386	364.6750	381.8711
36.00	430.3918	393.7316	457.9618	557.1123	232.5287	282.8742	264.5263	262.1476
48.00	336.8821	266.4340	411.0409	381.0683	153.3229	200.3760	170.0679	200.2678
72.00	174.5480	166.2749	246.2828	195.8913	59.1349	80.7777	78.5779	116.0798

TIME POINTS (Hrs)	**Subject-9**		**Subject-10**		**Subject-11**		**Subject-12**	
	Period-1	**Period-2**	**Period-1**	**Period-2**	**Period-1**	**Period-2**	**Period-1**	**Period-2**
0.00	BLQ	BLQ	no peak	BLQ	BLQ	BLQ	BLQ	BLQ
0.33	625.4278	103.6269	33.6109	168.6958	BlQ	499.4407	889.8900	770.6729
0.67	964.8641	1222.5468	248.2346	556.9724	BlQ	979.3912	858.7940	882.1268
1.00	754.1471	1185.5342	489.0155	715.0209	BlQ	821.3291	684.8525	885.9771
1.33	981.1961	1006.9688	780.0829	874.8497	28.6935	795.2971	1346.6967	843.1927
1.67	997.4247	1274.2224	815.8738	853.8506	72.5851	756.3153	892.6437	727.0715
2.00	831.1194	1193.7491	821.9824	847.1562	103.0993	684.1262	790.6754	706.6802
2.33	967.9029	1167.7938	883.4192	850.3795	143.7443	987.9591	987.7118	679.4585
2.67	1034.1361	1205.0387	954.7133	833.0704	149.6566	896.2199	810.5118	642.2839
3.00	817.7773	1201.4079	1037.5452	814.3912	551.1164	775.6969	1007.1974	874.3144
3.33	939.6493	1110.4027	901.3122	868.1038	668.2478	918.0450	924.7291	732.3955
3.67	860.6732	1053.3498	876.3602	859.0106	768.5237	827.6745	646.5832	849.9135
4.00	994.5000	1027.4403	977.1979	901.7499	737.8230	754.9582	994.7462	759.3361
5.00	891.1382	1003.6451	929.5762	793.6896	710.1949	667.0889	699.3830	688.0260
6.00	718.3462	955.7431	746.5370	901.6039	775.8195	618.1807	738.9122	704.6378
8.00	849.4305	1057.3754	769.1200	771.5552	715.5651	678.2709	873.8567	703.7738

10.00	734.5604	864.2511	734.8537	841.2455	697.0790	487.2798	738.9122	711.0259
12.00	745.2906	788.8073	663.5535	698.5064	628.0486	430.5915	760.5903	520.0823
16.00	571.7317	676.4503	554.6093	532.6121	592.7050	340.0950	475.9240	451.5706
24.00	490.6647	501.3881	486.1393	512.4101	421.0738	326.4136	478.2841	413.1769
36.00	273.5781	285.6498	287.6714	287.5680	242.2215	203.5685	258.4034	243.0551
48.00	absent	203.3989	232.2610	199.3008	184.1291	120.0949	259.6997	189.5752
72.00	86.7601	79.6356	81.7516	90.4294	73.7823	70.3990	absent	100.3022

9.0 Statistical methodology

There is a growing need of the application of mathematical statistics to a wide range of biological processes. Most people working in scientific research are forced to apply some concepts of biostatistics into their work if they want to share their results with the scientific community. This community needs to be convinced in order to trust your approach. And this is mostly done by biostatistics in forms of significance tests, confidence intervals and so on. A researcher is forced to show that his experimental outcome is not just a matter of chance for convincing other researcher to apply his methodology.

The following figure illustrates the statistical thinking process based on data in constructing statistical models for decision-making under uncertainties.

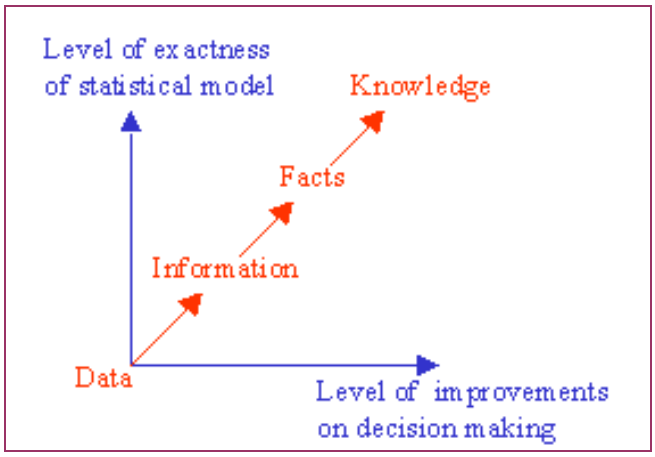


Figure - IV

The above figure depicts the fact that as the exactness of a statistical model increases, the level of improvements in decision-making increases. That's why we need statistical data analysis.

The post study data obtained is analyzed using various statistical tools like the **t-test, ANNOVA and the software like SAS and WinNonlin.**

Analysis Of Variance (ANOVA), a calculation procedure to allocate the amount of variation in a process and determine if it is significant or is caused by random noise. Using this statistical tool different parameters like the formulation effect, sequence affect, treatment effect between the test product and the reference product can be analysed.

WinNonlin is the industry standard for pharmacokinetic, pharmacodynamic, and noncompartmental analysis. In addition to its extensive library of built-in PK, PD and PK/PD models, WinNonlin supports custom, user-defined models to address any kind of data.

WinNonlin provides a complete solution with data management, statistical, modeling, and visualization tools in one package. Its worksheet interface facilitates data handling and transformations. Its descriptive statistics and linear mixed effects modeling engines provide flexible pre- and post-modeling analyses. The bioequivalence wizard supports

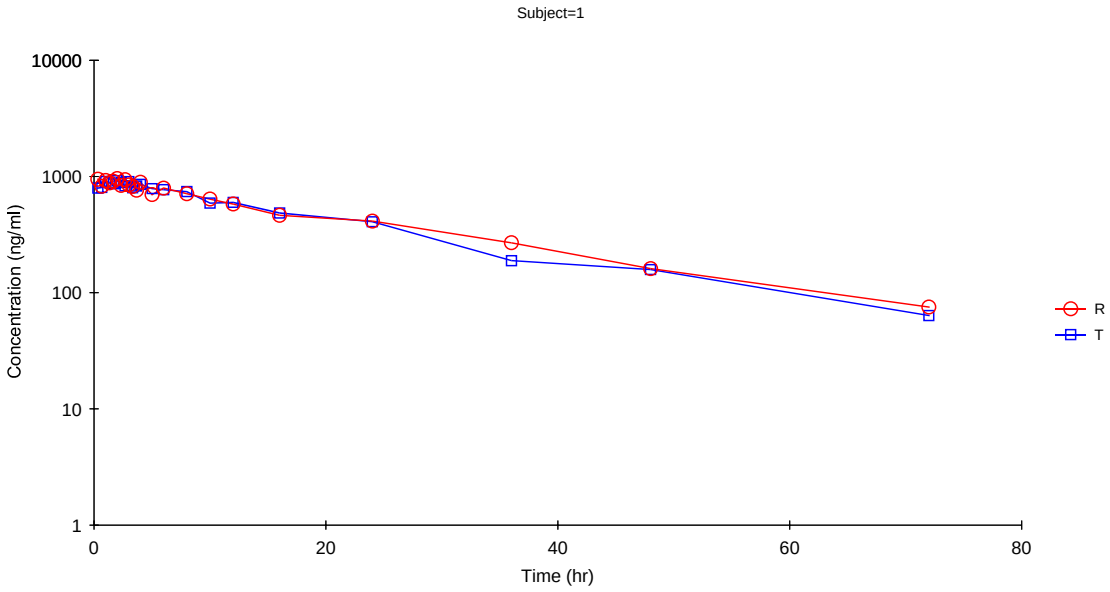
FDA standards for average, individual and population bioequivalence assessment. Additional tools enable exploration of your drug's properties through non-parametric superposition, semi compartmental modeling, deconvolution and nonparametric analysis of crossover studies. Finally, WinNonlin chart and table wizards, and automatic chart output from modeling, produce high-quality output for your study reports.

Statistical analysis software (SAS) - includes a vast range of statistics for general statistical analysis which includes multiple linear regression, correlation, ANOVA (analysis of variance), chi-square, Fisher, McNemar, Wilcoxon, Mann-Whitney, Friedman, Kruskal Wallis, Shapiro-Wilk normality tests, histograms, summary statistics and many more.

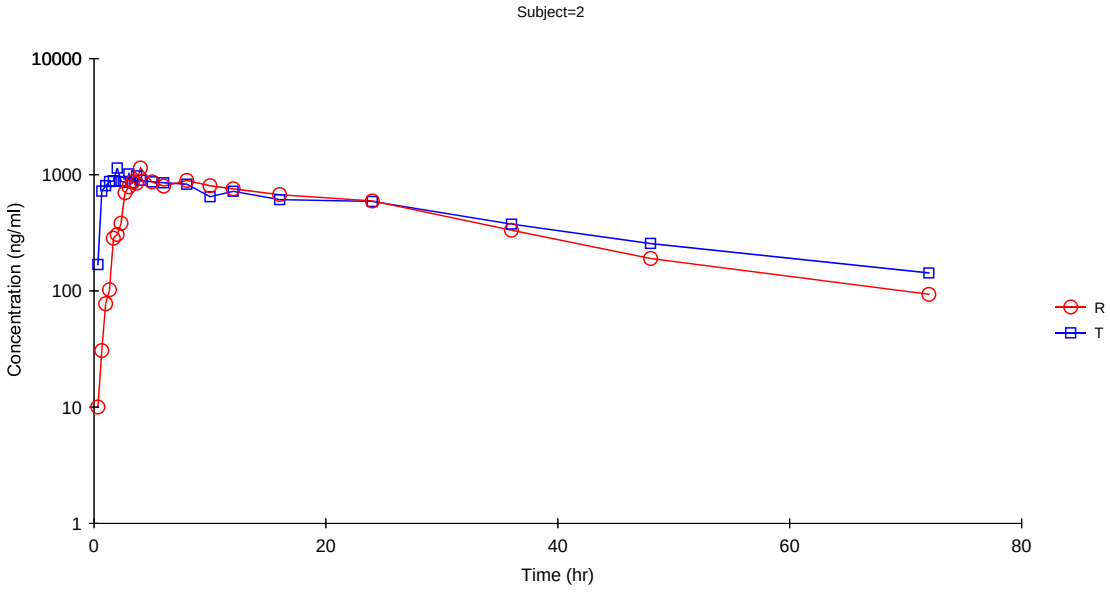
With the help of these software's the PK parameters and statistical results are calculated for the data obtained after the completion of the study.

RESULTS

Time Vs Concentration



Time Vs Concentration



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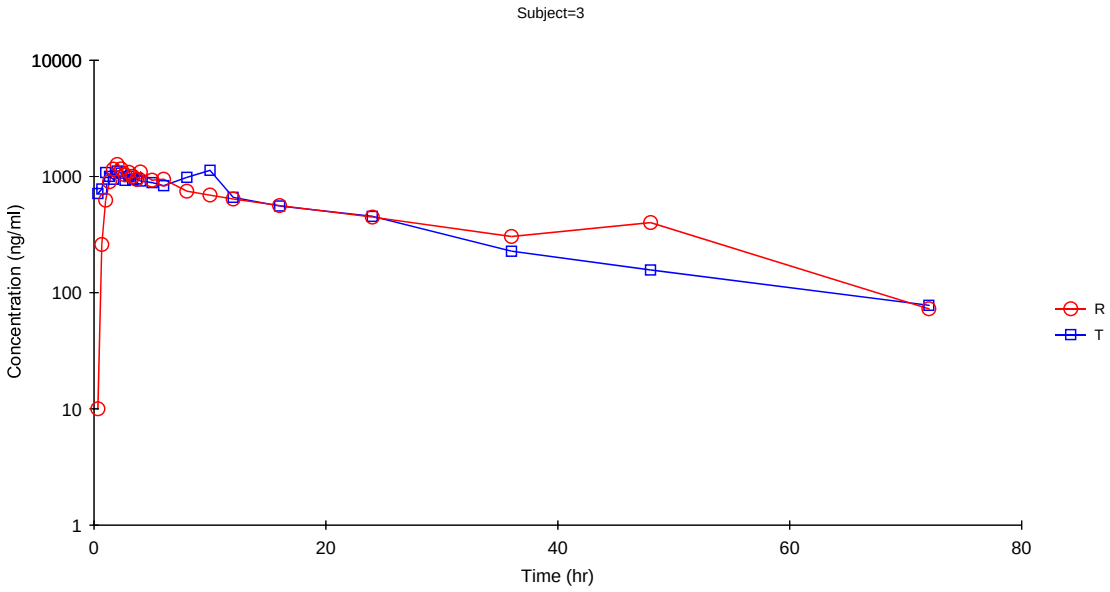

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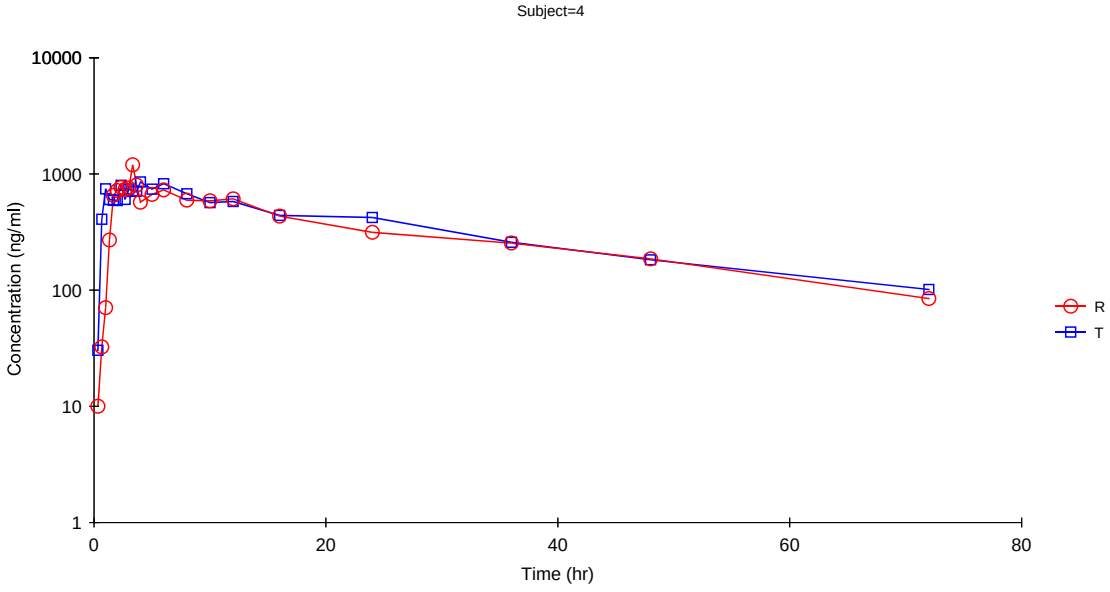

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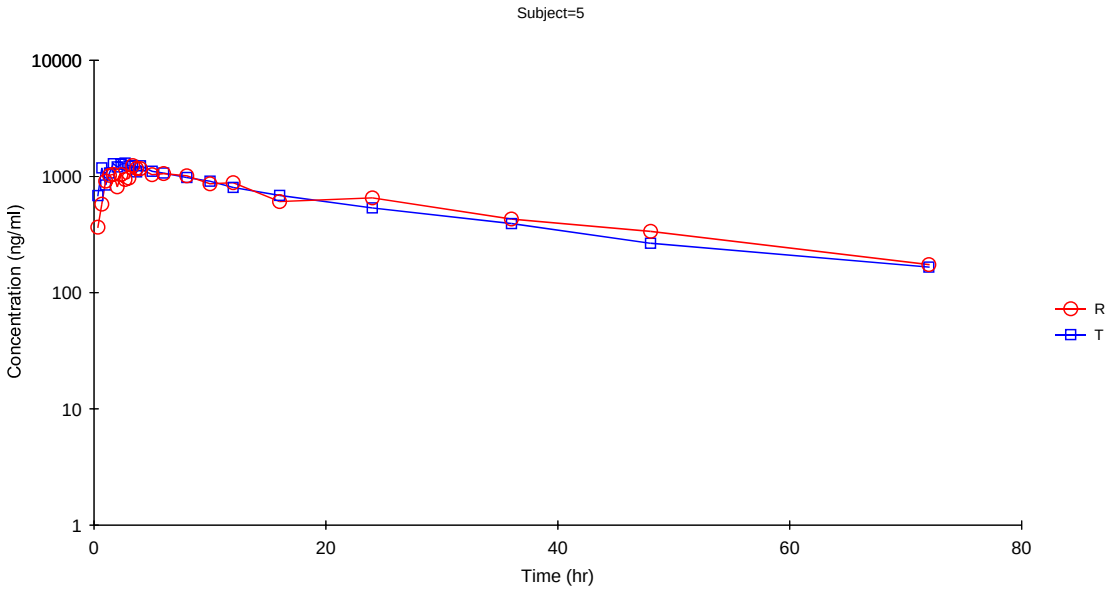
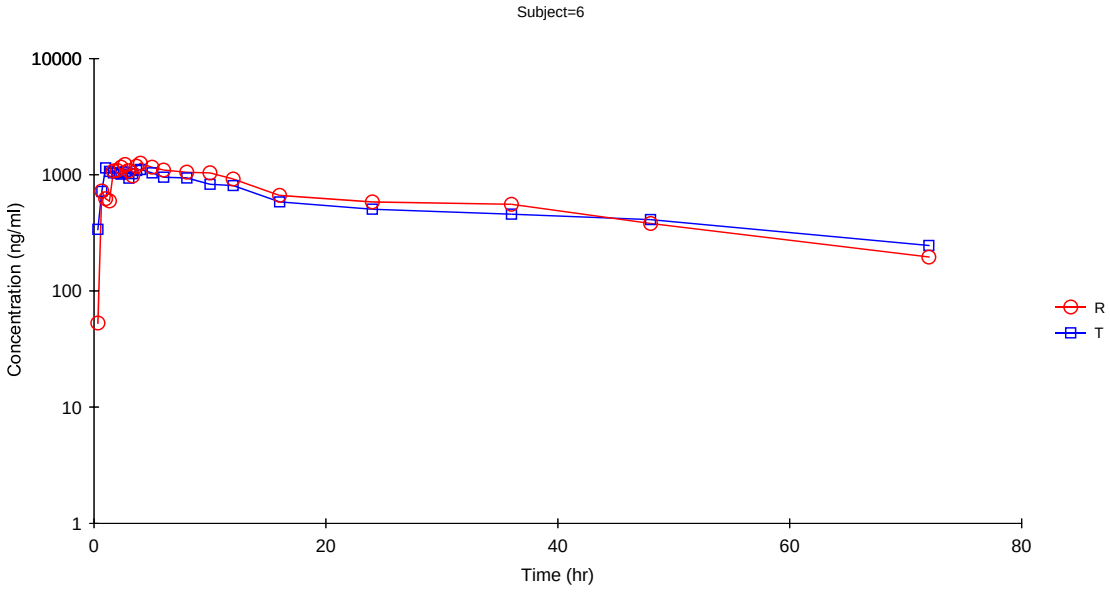


Time Vs Concentration



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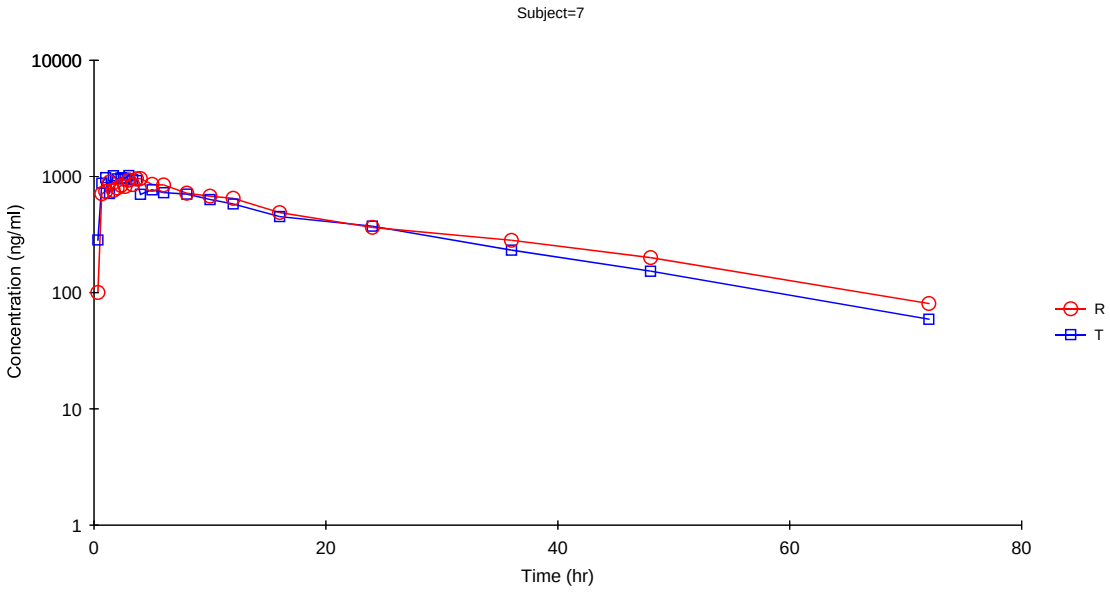
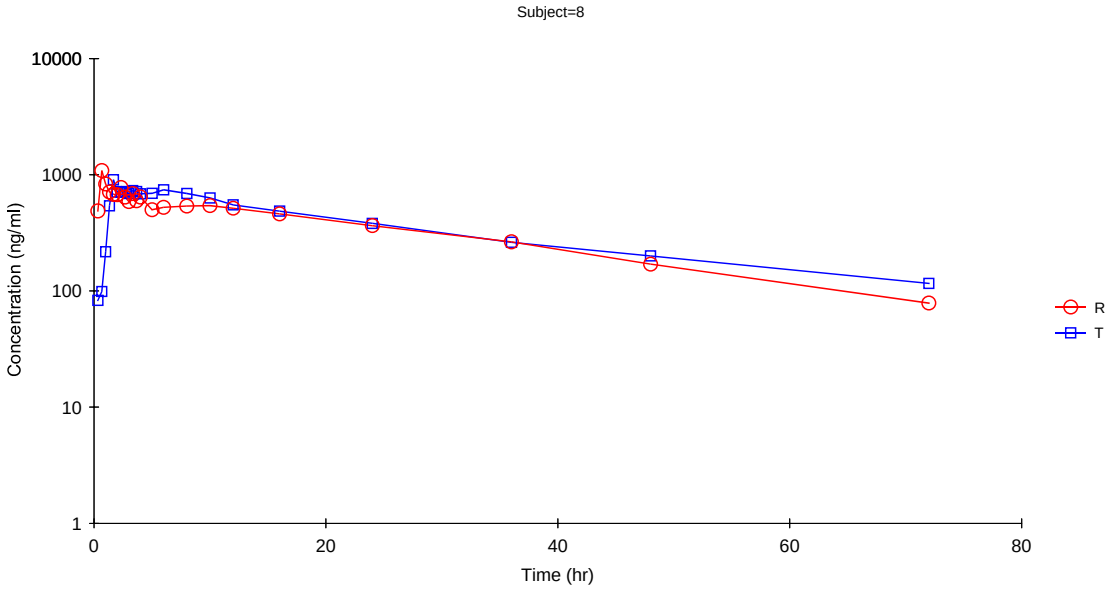


Time Vs Concentration



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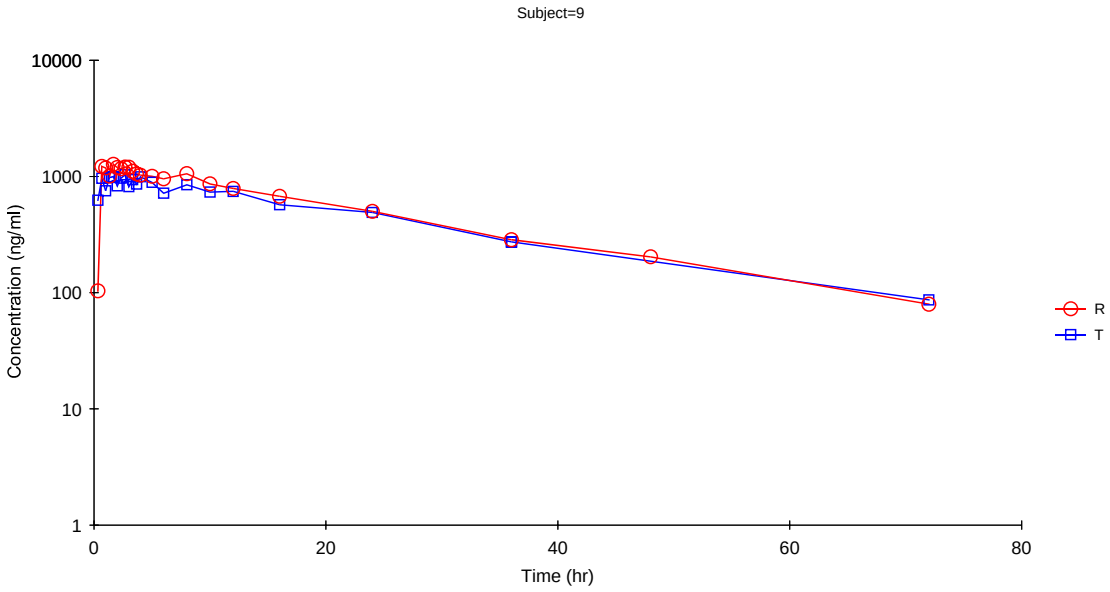

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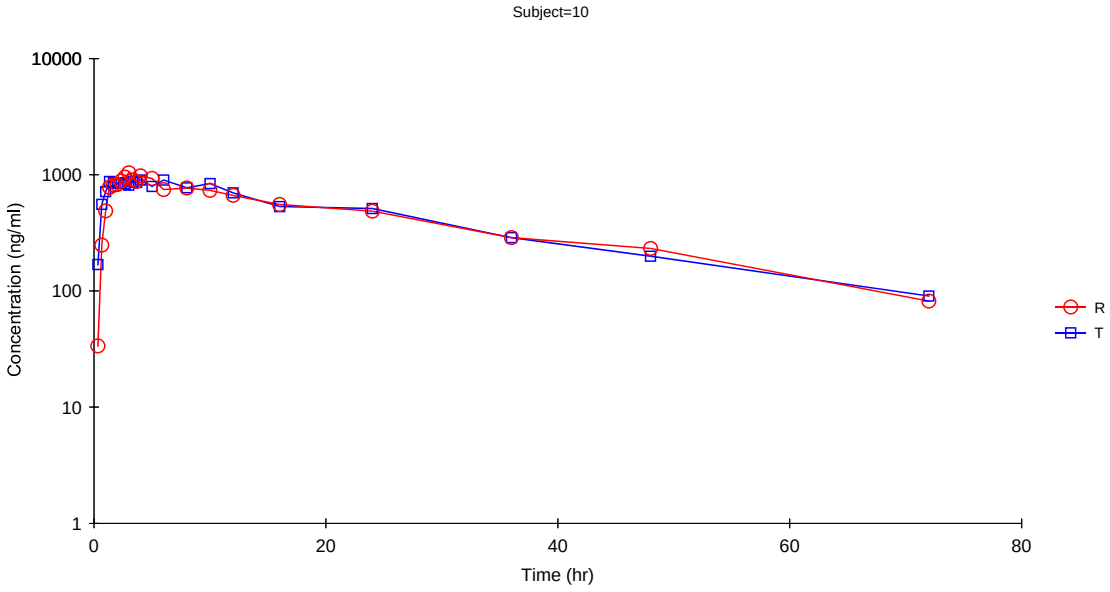

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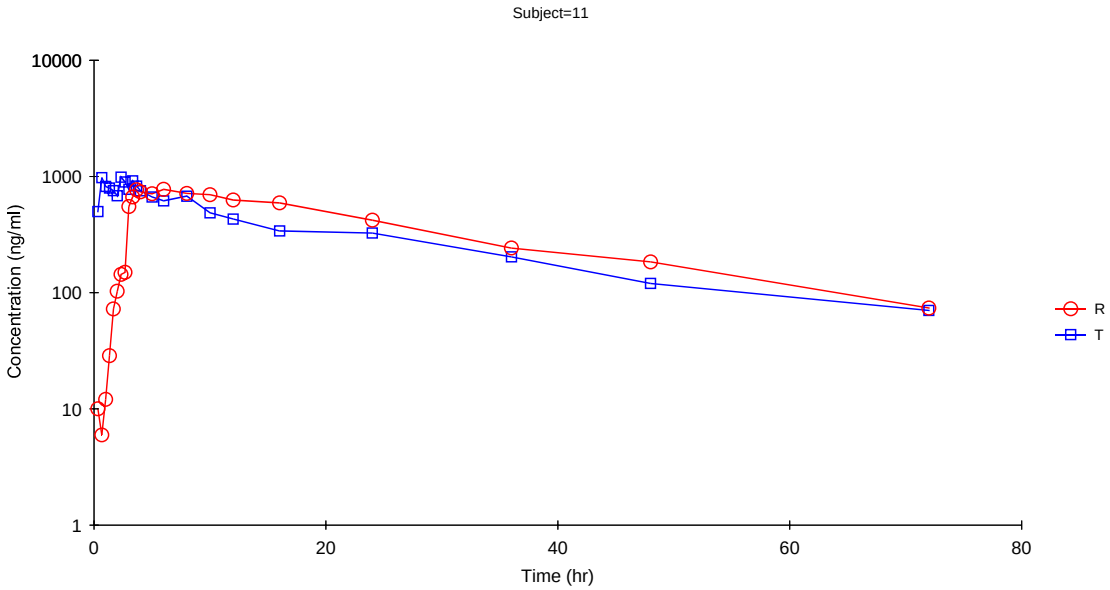
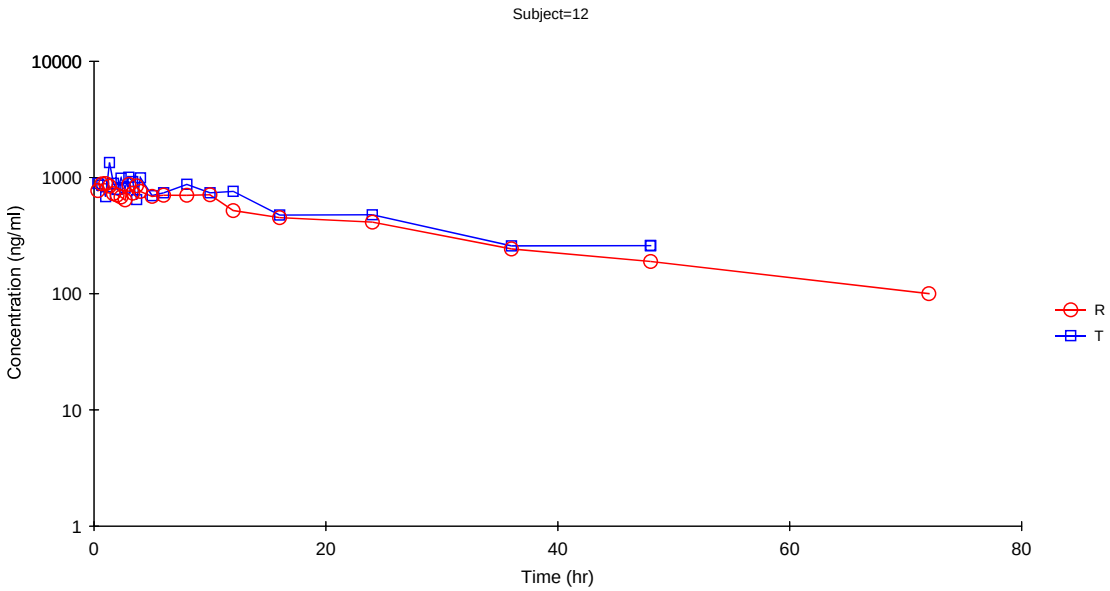
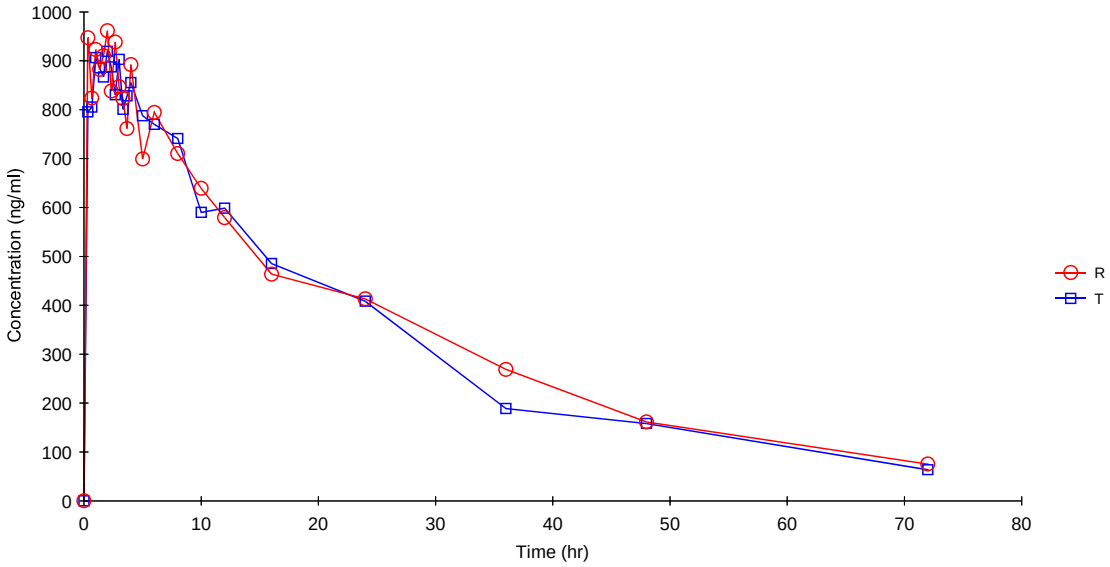


Time Vs Concentration



Time vs Cocentration

Subject=1



Time vs Cocentration

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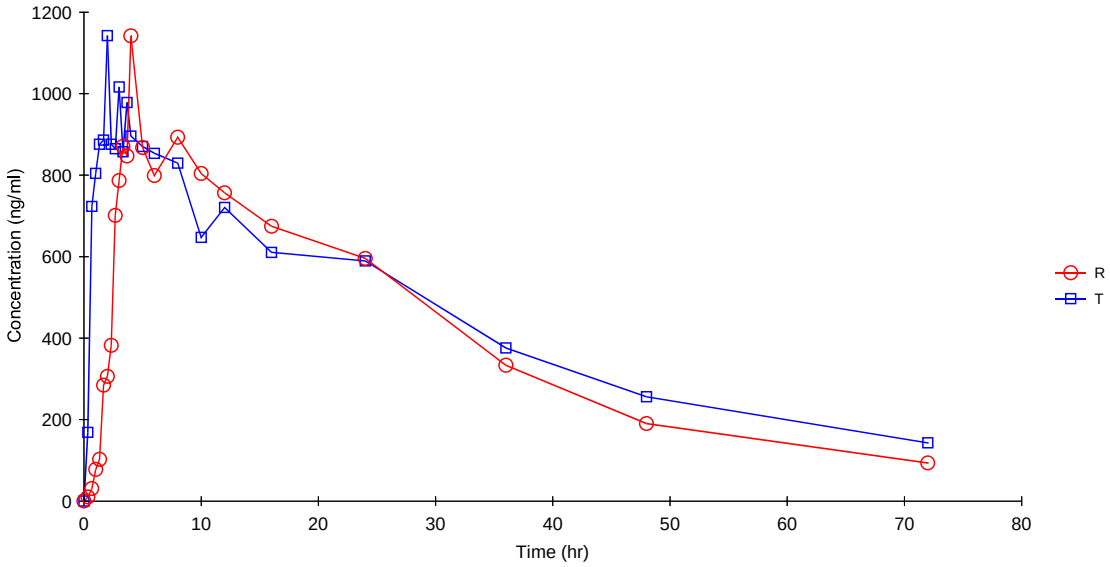

Time vs Cocentration

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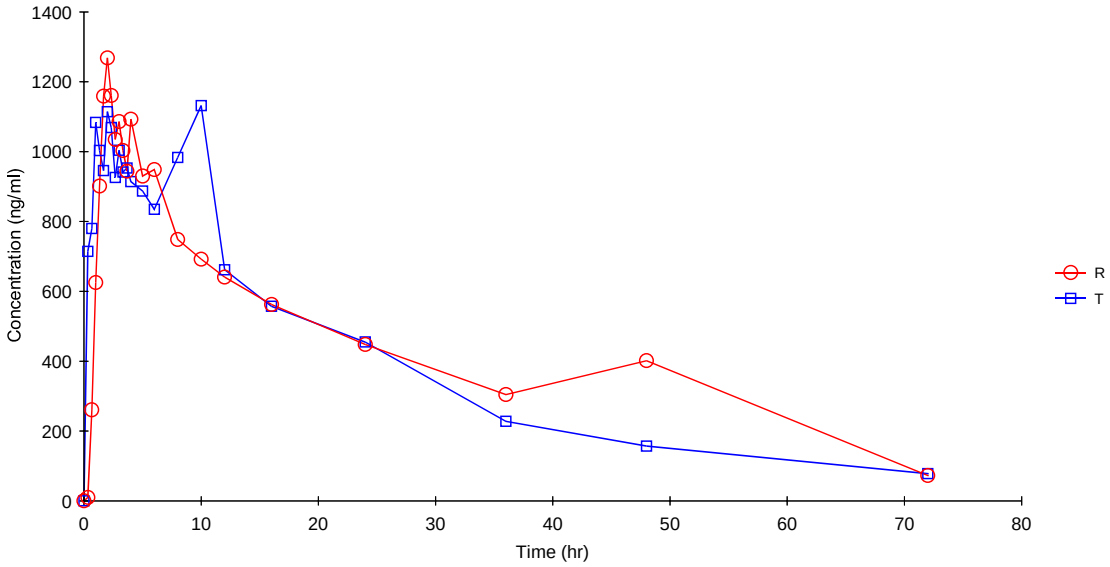

Time vs Cocentration

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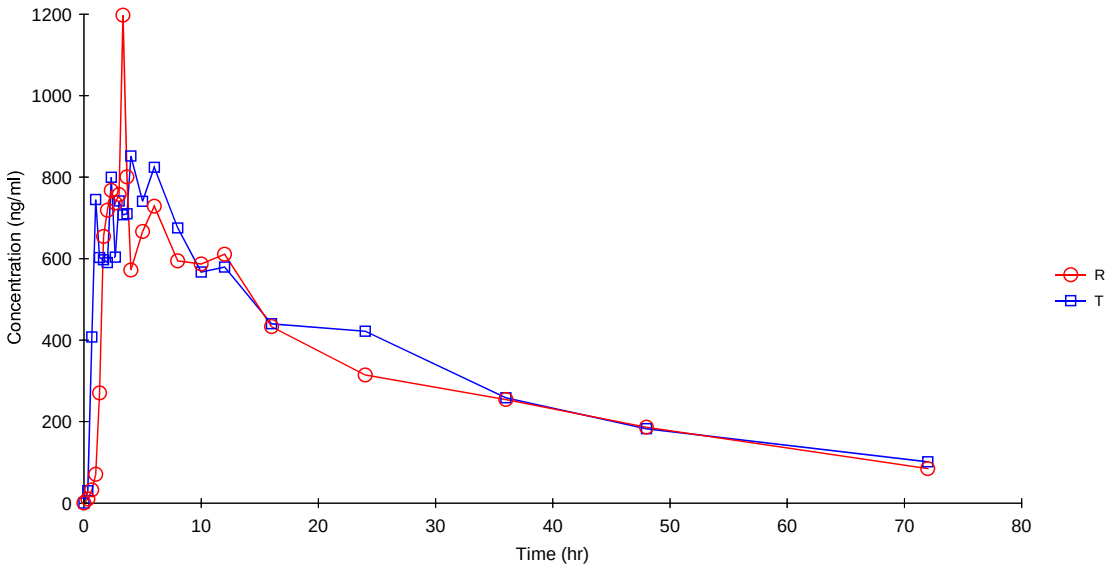
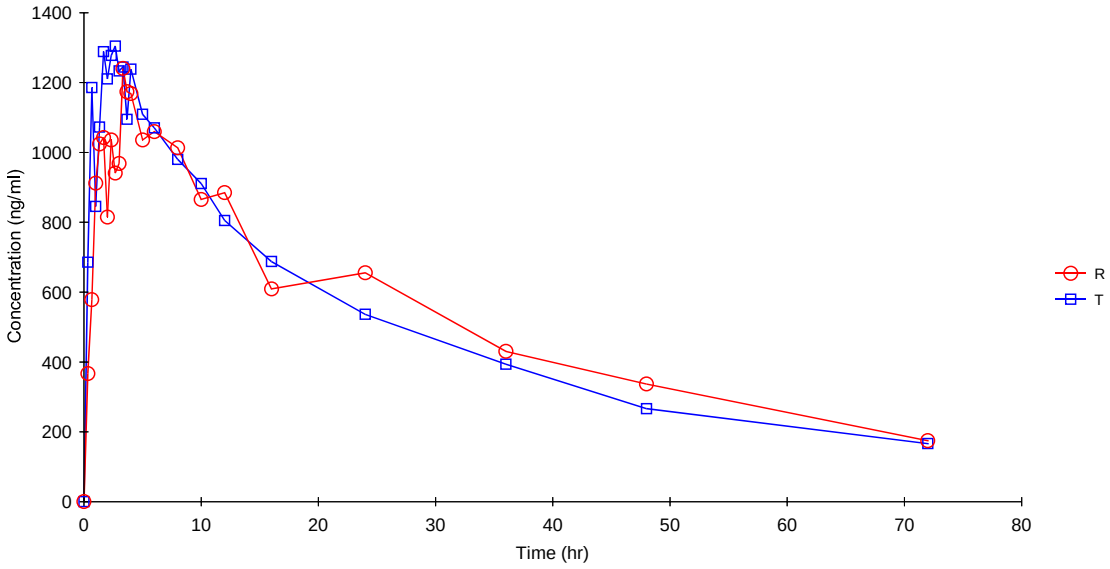


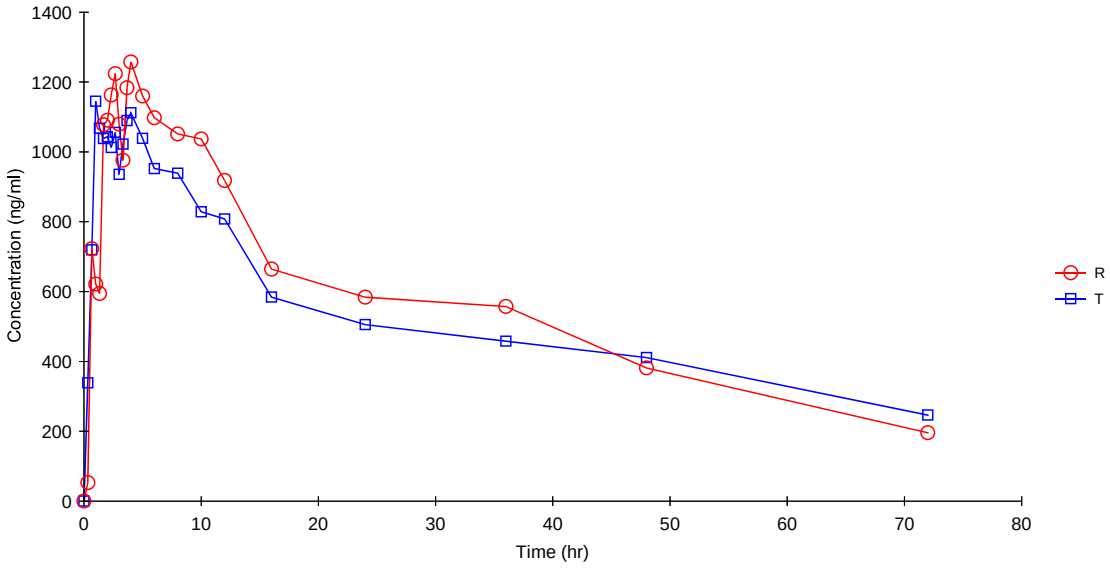
Time vs Cocentration

Subject=5



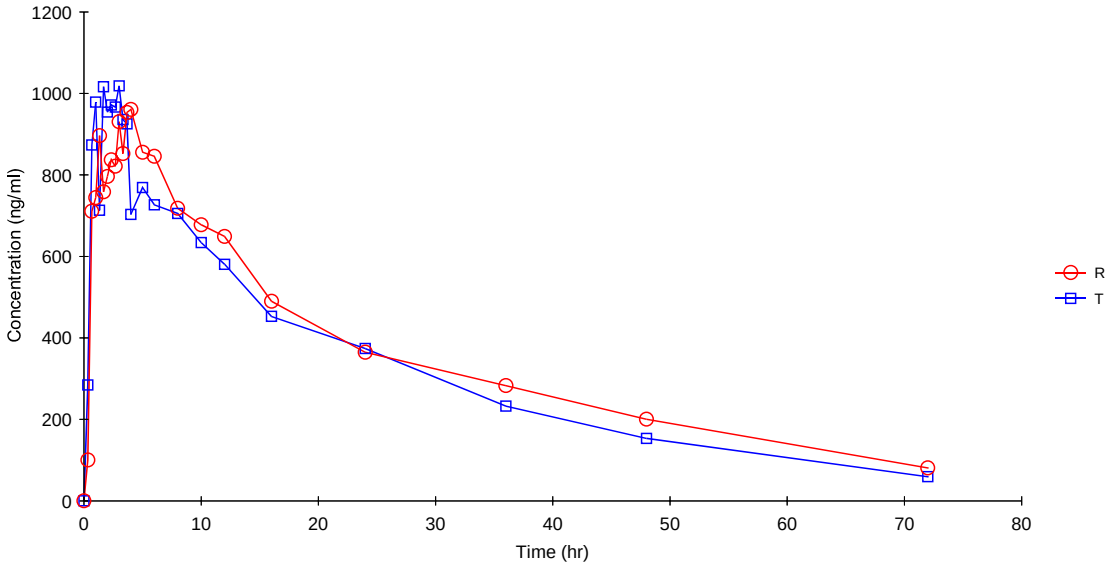
Time vs Cocentration

Subject=6



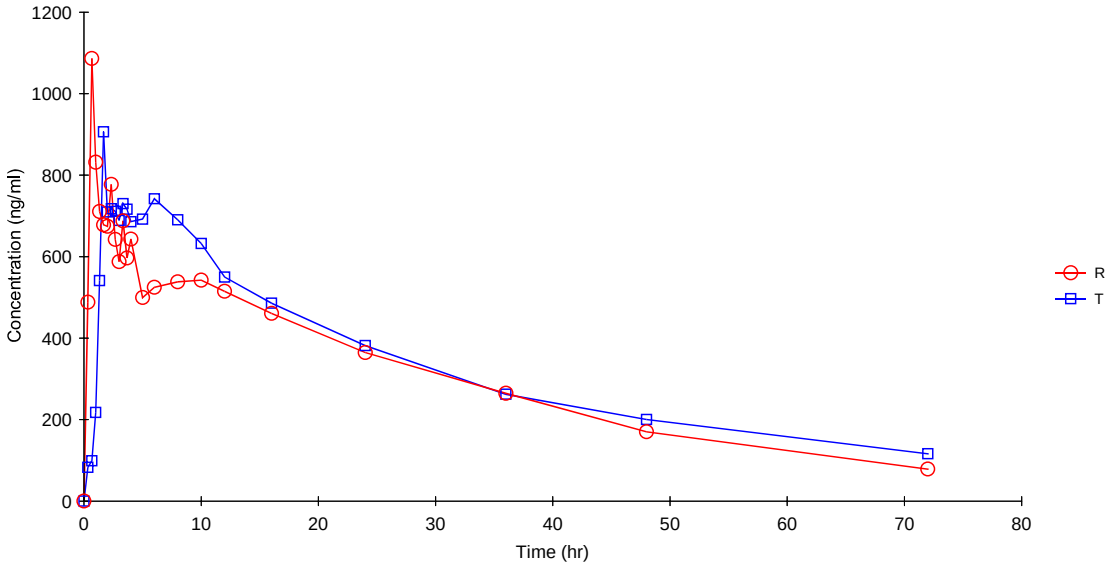
Time vs Cocentration

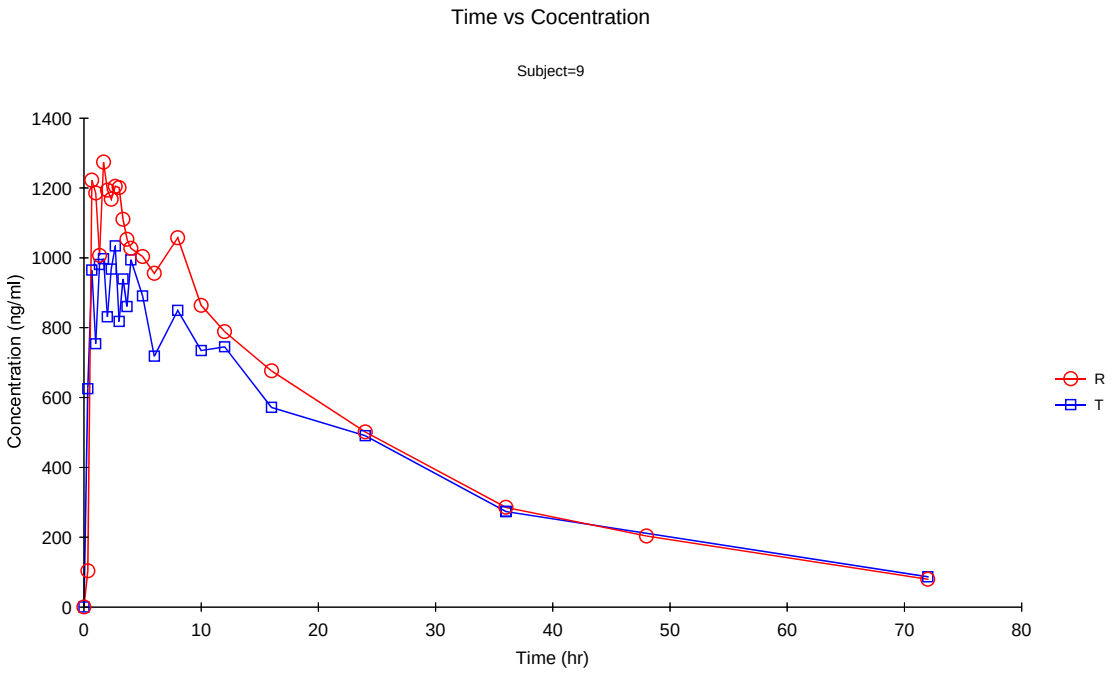
Subject=7



Time vs Cocentration

Subject=8




Time vs Cocentration
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Concentration (ng/ml)
Time (hr)
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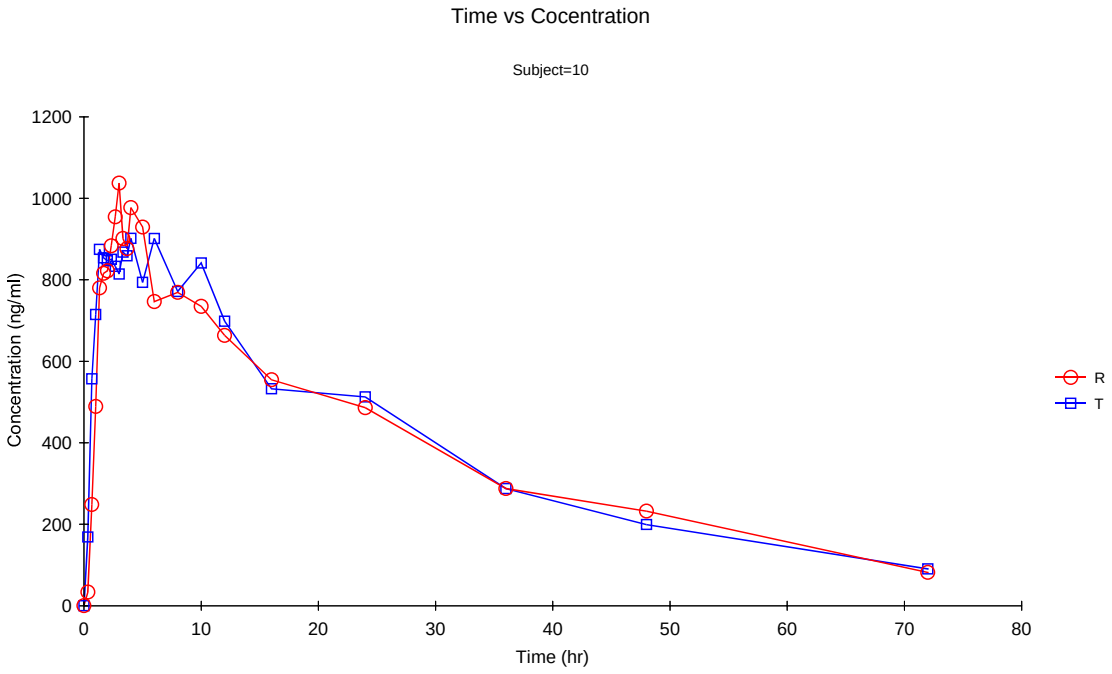
400
600
800
1000
1200
1400
0
10
20
30
40
50
60
70
80
R
T


Time vs Cocentration
Subject=10
Concentration (ng/ml)
Time (hr)
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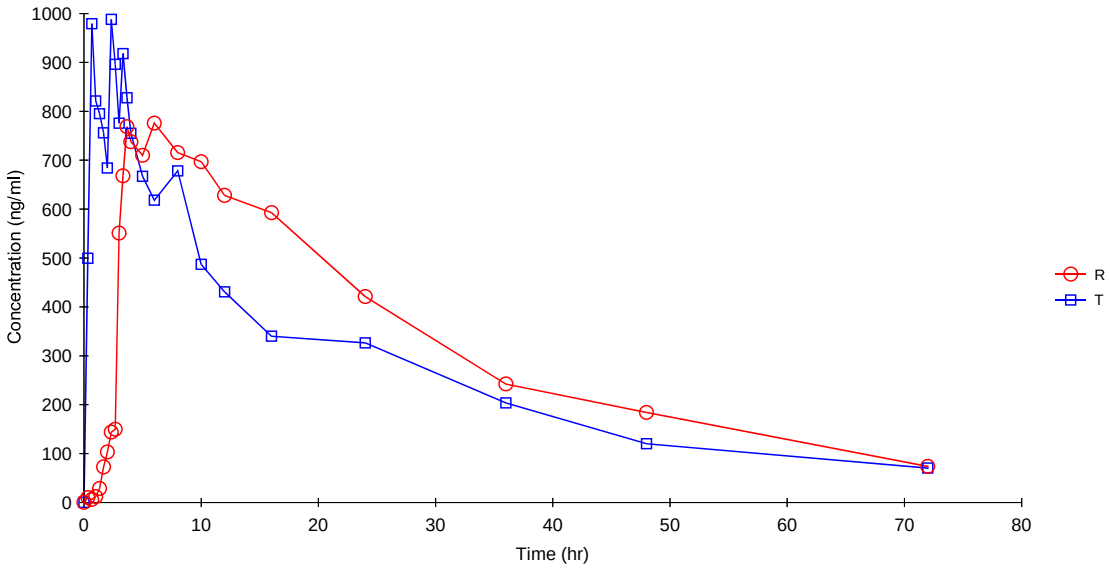
200
400
600
800
1000
1200
0
10
20
30
40
50
60
70
80
R
T

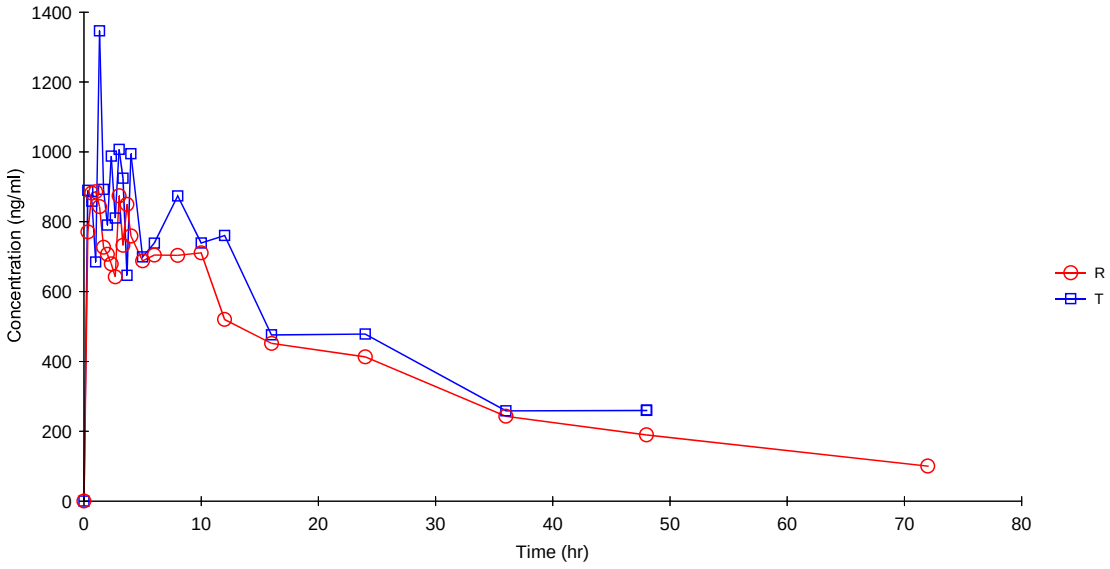
Time vs Cocentration

Subject=11



Time vs Cocentration

Subject=12



9.1 Pharmacokinetic parameters

Pharmacokinetic parameters and statistical analyses were calculated from subject concentrations using validated WinNonlin Version 5.1 and SAS Version 9.1 software procedures, respectively.

Pharmacokinetic Data

PK Parameter results based on untransformed data for drug in 12 subjects (Fasting)

Table – XVI

Treatment	Subject	Lambda_z	HL_Lambda_z	Tmax	Cmax	AUC_{last}	AUC_{INF}	Period	Sequence
R	1	0.0357	19.438	2	961.5539	24092.21	26203.18	2	TR
R	2	0.0359	19.3024	4	1141.68	28513.34	31118.87	1	RT
R	3	0.0345	20.106	2	1268.56	30638.97	32747.16	2	TR
R	4	0.0309	22.4652	3.33	1197.851	21502.83	24247.03	1	RT
R	5	0.0269	25.7391	3.33	1240.501	36715.47	43197.09	1	RT
R	6	0.0288	24.0286	4	1257.688	39560.91	46351.68	2	TR
R	7	0.0352	19.6647	4	960.7402	24861.6	27153.29	2	TR
R	8	0.0335	20.6928	0.67	1086.579	21599.99	23945.81	1	RT
R	9	0.0384	18.0636	1.67	1274.222	30357.22	32432.55	2	TR
R	10	0.0339	20.4191	3	1037.545	27116.03	29524.3	1	RT
R	11	0.0359	19.309	6	775.8195	22786.1	24841.45	1	RT
R	12	0.0249	27.8786	1	885.9771	23880.31	27914.49	2	TR
T	1	0.0385	18.0038	2	919.2609	22989.55	24640.87	1	TR
T	2	0.0265	26.1638	2	1142.762	31320.06	36714.33	2	RT
T	3	0.0297	23.3296	10	1131.794	26856.85	29477.96	1	TR
T	4	0.0258	26.8887	4	851.7415	23406.31	27337.73	2	RT
T	5	0.0264	26.2513	2.67	1304.585	34893.21	41190.48	2	RT
T	6	0.0147	47.1075	1	1145.174	37065.03	53802.83	1	TR
T	7	0.0382	18.1237	3	1018.557	22674.87	24221.07	1	TR
T	8	0.0226	30.613	1.67	906.5869	23477.27	28603.96	2	RT
T	9	0.0355	19.5458	2.67	1034.136	27760.83	30207.35	1	TR
T	10	0.0322	21.4937	4	901.7499	27259.06	30063.17	2	RT
T	11	0.0313	22.1221	2.33	987.9591	19457.3	21704.12	2	RT
T	12	0.0307	22.6087	1.33	1346.697	23558.65	32029.41	1	TR

9.2 Statistical Data

The statistical results obtained for the drug during the fasting study were as follows:

9.2.1 PK Parameter results based on untransformed data for drug in 12 subjects (fating)

Table – XVII

Treatment		Cmax	Tmax	$AUC_{0\text{-last}}$	$AUC_{0\text{-INF}}$	HL_Lambda_z	Lambda_z
R	**Mean**	1090.726	2.920	27635.414	30806.407	21.426	0.033
	SD	167.112	1.520	5831.224	7210.528	2.996	0.004
	CV%	15.321	52.030	21.101	23.406	13.983	12.505
	Geometric Mean	1078.223	2.490	27117.472	30129.766	21.248	0.033
T	**Mean**	1057.584	3.06	26726.5826	31666.1058	25.1876	0.0293
	SD	159.6985	2.38	5316.2684	8780.0679	7.8704	0.0068
	CV%	15.1003	77.81	19.8913	27.727	31.2471	23.0449
	Geometric Mean	1046.904	2.54	26271.4831	30720.9243	24.3099	0.0285

9.2.2 Summarized statistical results of Primidone based on ln-transformed data in 12 subjects (Fasting)

Table – XVIII

Parameter*		**C_{max} (ng /mL)**	**AUC_{0-t} (ng hr/mL)**	**$AUC_{0-\infty}$ (ng.hr/mL)**
Geometric LSM	**T** **R**	1046.9038 1078.2232	26271.4831 27117.4723	30720.9243 30129.7662
Geometric LSM Ratio	**T/ R**	97.10	96.88	101.96
90% Confidence Interval: T Vs. **R**	Lower Limit Upper Limit	86.86 % 108.54 %	93.09 % 100.83 %	95.58 % 108.77 %
p-values (ANOVA):	Sequence Period Treatment	0.4701 0.6480 0.6746	0.6590 0.7246 0.7873	0.6701 0.8257 0.8877
Intra-subject Variability:		15.14	5.40	8.75
Power (%):		95.06	100	99.94

The details of the ratios of means of test and reference parameters such as In-transformed C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ of **Test** and **Reference** at 90% confidence interval were as follows:

Table – XVIII

Parameter	**Ratio (%)**	**Lower limit**	**Upper limit**
C_{max}	97.10	86.86	108.54
AUC_{0-t}	96.88	93.09	100.83
$AUC_{0-\infty}$	101.96	95.58	108.77

DISCUSSION

The present thesis was subdivided in seven chapters, in these,

In chapter 1- I had discussed about the bioserve clinical research company

In chapter 2 - I had discussed about the introduction about bioavailability, bioequivalence, and epilepsy etc.

In chapter 3 - I had discussed about the main Objectives of the present study.

In chapter 4 - I had discussed about drug profile of Primidone, its various pharmacokinetic and pharmacodynamic parameters

In chapter 5 - I had discussed about Design and conduct of the study for the execution of the protocol and various parameters to be investigated

In chapter 6 - I had discussed about the clinical phase of the study. This covers various aspects of patient related factors of the study.

In chapter 7 - I had discussed about the Bioanalytical methods to characterize the presence of drug in the plasma.

In chapter 8 - I had discussed about the various statistical methods and the results of the present study.

Table no. 1 - Discusses Marketed Drugs Used to Treat Seizures

Table no 2 -Discusses SUBJECT DEMOGRAPHICS

Table no 3 - Discusses Total volume of blood collected from each subject during the Study.

Table no 4 - Discusses Laboratory Parameter Investigations

Table no 16 - Discusses PK Parameter results based on untransformed data for drug in 12 subjects (Fasting)

Table no 17 - Discusses statistical results obtained for the drug during the fasting study

Table no 18 - Discusses Summarized statistical results of Primidone based on ln-transformed data in 12 subjects (Fasting)

Table no 19 - Discusses Details of the ratios of means of test and reference parameters such as In-transformed $C_{max,}$ $AUC_{0-t,}$ $AUC_{0-\infty}$ of Test and Reference at 90% confidence interval

Glossary of terms

1. Adverse Event: An adverse event is any untoward medical occurrence in clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.

2. Analysis of Variance (ANOVA): ANOVA is a statistical technique to identify sources of variance and estimate the degree of variability. In most bioavailability studies, there are three readily identified sources of variance namely formulation (Treatment), subject and period; hence it is a 3-way ANOVA.

3. Area under the curve (AUC): Area under the curve is the total area under the biological fluid (serum, blood, etc.) concentration-time curve as determined by the Trapezoidal rule.

4. AUC_Extrapolated (%): Calculated as {1 - (AUC0-t / AUC0-∞)} x 100. The mean AUC_Extrapolated (%) should be ≤ 20%.

5. Bioavailability studies: It involves the determination of 'Drug' concentration in the blood or urine. Concern with how quickly and how much of a 'Drug' appears in the blood after a specific dose is administered.

6. C_{max}: This is the maximum ‘Drug’ concentration achieved in systemic circulation following ‘Drug’ administration

7. Good Clinical Practice: A standard for the design, conduct, performance, monitoring, auditing, recording, analyses and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected.

8. Informed Consent: A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial are relevant to the subject’s decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form.

9. Investigational Product: A pharmaceutical form of an active ingredient being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.

10. K_{el}: Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve, using the method of least square regression.

11. Protocol: A document that describes the objective(s), design, methodology, statistical consideration, and organization of a trial. The protocol usually also give the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the ICH-GCP Guidance, the term protocol refers to protocol and protocol amendments.

12. Quality Assurance: All those planned and systematic action that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with GCP and the applicable regulatory requirements(s).

13. Quality Control: The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial related activities have been fulfilled.

14. Randomization: The process of assigning trial subject to treatment groups using an element of chance to determine the assignments in order to reduce bias.

15. Sponsor-Investigator: An individual who both initiate and conducts, alone or with others, a clinical trial and under whose immediate direction the investigational product is administered to, dispensed to, or used by a subject. The term does not include any person other than an individual (e.g. it does not include a corporation

or an agency). The obligations of a sponsor-investigator include both those sponsor and those of an investigator.

16. T_{max}: It is the time required to achieve maximum 'Drug' concentration in systemic circulation

17. $t_{1/2}$: Terminal half-life as determined by quotient 0.693/ Kel

CONCLUSION

All the study procedures followed were in compliance with the protocol, the ICH-GCP guidelines and Schedule Y.

From the analyses of pharmacokinetic and statistical results it was inferred that, for the ln-transformed data, the 90 % confidence interval about the test to reference ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of drug Primidone were falling within the bioequivalence acceptance range of 85.00 % - 109.00 %, which demonstrates the bioequivalence of test formulation 'T' with reference formulation 'R' under fasting conditions.

From the clinical data it can be concluded that the study objectives like the safety and efficacy of the test product has been achieved.

APPENDIX-I

ACTIVITIES SCHEDULE

Study phase	**Screening** With in 28 days of dosing	Check-in day	Dosing day
Activity			
Informed consent	X	X	
Medical history and demographic data	X		
Physical examination	X		
ECG (12-lead)	X		
Vital signs measurement	X		
Hematology	X		
Urine analysis	X		
Clinical chemistry	X		
Serology(HIV-I&II, hepatitis B&C, RPR)	X		
Urine drug screening ethanol, benzodiazepines, cannabionoids, amphetamines, cocaines, opiates (period-I)		X	
Record of concomitant medication		X	X
Check in procedures		X	

Alcohol screening(periods I&II)	X	X	
Confinement in study unit		X	X
Drug dosing			X
PK blood sampling			X
Adverse events monitoring		X	X

APPENDIX-II

Pre-Study Laboratory Safety Evaluation Parameters

Clinical chemistry

S.No.	PARAMETER	REFERENCE INTERVALS	UNITS
1	Random blood glucose	70 to170	mg/dl
2	Urea	15 to 40	mg/dl
3	Creatinine	0.6 to 1.5	mg/dl
4	Total bilirubin	0 to 1.0	mg/dl
5	SGPT(ALT)	30 to 65	U/L
6	SGOT(AST)	15 to 37	U/L
7	Alkaline phosphatase(ALP)	50 to 136	U/L
8	Albumin	3.7 to 5.3	g/dl
9	Sodium	136 to149	mEq/L
10	Potassium	3.8 to 5.2	mEq/L

SEROLOGY

SEROLOGY SCREENING	INDICATION
HIV-1&2	HIV ANTIBODIES AIDS
Abs antigen& HCV	HEPATITS B& HEPATITIS C VIRUS
RPR Test	SYPHILIS

HAEMATOLOGY

S.No.	PARAMETER	REFERENCE INTERVALS	UNITS
1	Total W.B.C	4000 to 1100	/cmm
2	Total R.B.C	4.5 to 6.5	mil/cu mm
3	Hemoglobin	14 to 18	gms%
4	HCT(PCV)	40 to 54	Vol%
5	Neutrophils	40 to 75	%
6	Lymphocytes	20 to 40	%
7	Eosinophils	01 to 06	%
8	Monocytes	2 to 8	%
9	Basophils	0 to1	%
10	Platelets	104 to 400 x 10^3	/cmm
11	ESR(1^{st} hr)	0 to 15	mm

URINE ANALYSIS

PARAMETER
Physical examination
Color
Transparancy
Specific gravity
pH
Chemical examination
Glucose
Protein
Ketones
Blood
Urobilinogen
Bilirubin
Microscopical examination
Pus cells
RBC’s
Epithelial cells
Crystals
Casts
others

APPENDIX-III

MEAL MENU FOR SUBJECTS PARTAKING

IN THE PRIMIDONE STUDY

Pre-study dinner	(Standard meal)Rice with one curry, dal, sambar, rasam, curd and papad
Break fast	----------
Lunch	Standard meal
Snacks	Two somosa
Dinner	Standard meal

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